DOCTORAL THESIS

Modulation of sensory and pain perception with successive non-invasive brain stimulation

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Modulation of sensory and pain perception with successive non-invasive brain stimulation

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Abstract

Introduction: Non-invasive brain stimulation techniques are being trialed to induce neuroplasticity for meaningful purposes. Transcranial direct current stimulation (tDCS) is one such brain stimulatory technique, which involves delivering low amplitude direct current (1-2mA) to the brain via scalp electrodes. A review of the literature has suggested that repeated daily tDCS could induce lasting effects in the motor domain in a healthy population and in both the sensory and motor domains in a clinical population (Boggio et al. 2007, Mori et al. 2012, Reis et al. 2009). Of interest was whether increasing tDCS dose could evoke cumulative body sensory system function alteration in a healthy population.

Aims: A systematic review aimed to review the literature most relevant to 1_the effects of sensory cortex tDCS on sensory threshold related outcome measures and 2_the effects of motor cortex tDCS on pain threshold/intensity related outcome measures. Study 1 aimed to investigate the effects of consecutive daily sessions of tDCS on a sensory psychophysical outcome measure in a healthy population. Study 2 aimed to investigate the effects of consecutive daily sessions of tDCS on a series of pain related psychophysical, subjective and objective outcome measures in a healthy population as well as investigate the correlation between the baseline pain related psychophysical, subjective and objective outcome measures in a healthy population.

Methods: A systematic review of the literature most relevant to the aims of studies 1 and 2 was firstly undertaken. Randomised controlled trial

methodology was then utilised in Study 1 to assess the effects of 5 consecutive daily sessions of active (anodal) or sham sensory cortex tDCS on one psychophysical (i.e. vibration detection thresholds) measure in 29 healthy human volunteers. In Study 2, randomised controlled trial methodology was used to assess the effects of 5 consecutive daily sessions of active (anodal) or sham motor cortex tDCS on psychophysical (i.e. electrical, mechanical pressure and thermal detection and pain thresholds), subjective (i.e. electrical, thermal and mechanical pressure pain visual analogue scales (VAS)) and objective (i.e. salivary cortisol) outcome measures in 42 healthy human volunteers. Cross-sectional analysis of baseline data was also used in Study 2 to explore bivariate correlations between examined outcome measures.

Results: The review indicated both methodological limitations and heterogenous tDCS induced effects for trials. The review also revealed that repeated stimulation was one area that researchers had failed so far to focus on. Studies 1 and 2 demonstrated that consecutive daily sessions of anodal tDCS could not consistently alter psychophysical, subjective and objective outcome measures compared to sham in a healthy population. Study 2 also demonstrated statistically significant correlations between psychophysical and subjective outcome measures in a healthy population.

Conclusion: The results of studies 1 and 2 suggest that increasing tDCS dose does not result in more consistent anodal tDCS induced effects on body sensory/pain perception in a healthy population. As well, the results of Study 2 also may provide further evidence of the clinical utility of different types of pain assessments.

Declaration of original work

This thesis is submitted to Bond University in fulfillment of the degree of Master of Philosophy. This thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this University or any other institution, except where due acknowledgement is made.

Signed:

Brookes Gregory Folmli

Date:

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List of acronyms

ANOVA – analysis of variance

BDNF - brain-derived neurotrophic factor

CPT - cold pressor pain threshold

CTT - cold pressor tolerance threshold

EDT - electric detection threshold

EEG - electroencephalography

ELISA – enzyme-linked immunosorbent assay

EP – experimental pain

EPT - electric pain threshold

EMG- electromyography

FDI – first dorsal interosseous

fMRI – functional magnetic resonance imaging

GABA – gamma-amino-butyric acid

HPA - hypothalamic-pituitary-adrenal

LTP – long-term potentiation

MEP – motor evoked potential

MOR - mu opioid receptor

MRI – magnetic resonance imaging

MRS - magnetic resonance spectroscopy

NaCl – sodium chloride

PPT - pressure pain threshold

rTMS – repetitive transcranial magnetic stimulation

SD – standard deviation

SEP - somatosensory evoked potentials

SPSS – statistical package for the social sciences

SRGPSQ - self-reported general pain sensitivity questionnaire

tDCS - transcranial direct current stimulation

TMS – transcranial magnetic stimulation

TrkB – tropomyosin related kinase B

VAS – visual analogue scale

VDT - vibration detection threshold

Chapter 1

Introduction & Literature review

In line with the research goals of this thesis, this section will first briefly review the history, principle, key variables, key limitations and standard delivery parameters of a non-invasive nervous system stimulation technique, namely transcranial direct current stimulation (tDCS). This section will then briefly review clinical and research applications for tDCS in the sensory and motor domains. Finally, this section will look at postulated mechanisms for tDCS induced post stimulation effects. Further critical review of the relevant literature relating to the potential effects of tDCS on sensory/pain perception in a healthy human population is presented in a systematic review, which is presented in Chapter 2.

1.1 The history of tDCS

Transcranial application of weak direct currents to the human brain was initially performed in the 1960's, trialed mainly on patients living with psychiatric disorders. These preliminary studies revealed that stimulation could improve either depressive or manic symptoms (Costain, Refearn & Lippold 1964, Carney 1969).

Further research in the 1980's revealed lasting tDCS induced effects on choice reaction time tasks in a healthy population (Jaeger et al. 1987). Despite this, interest with the device seemingly faded, which may have been the result of a lack of methods to monitor its effects beyond the phenemological level (Wassermann et al. 2008).

The ability to conveniently monitor induced changes to brain activity with techniques like transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI), however, has helped to validate the tDCS technique and seemingly spark renewed interest with the device (Nitsche, Paulus 2000). Currently, tDCS has been shown to affect a variety of functions in healthy humans and neuropsychiatric conditions (Flöel 2014, Shin, Foerster & Nitsche 2015).

1.2 The principle of tDCS

A constant direct current is delivered to the brain in tDCS, which is caused by asserting the two poles of an electric battery to the brain (Kropotov 2009). However, tDCS is not able to induce neuronal action potentials. This rests largely on the fact that the static uniform extra-cellular electric fields that are present in tDCS simply cannot yield the rapid depolarization required for this to occur (Nitsche et al. 2008). Instead, tDCS affects neuronal activity through alteration of the transmembrane potential of exposed brain parenchyma, causing either tonic depolarisation or hyper-polarisation of the tissue's membrane (Creutzfeldt, Fromm & Kapp 1962, Purpura, McMurtry 1965). This action consequently leads to an alteration of both the level of excitability and firing rate of the neurons affected. The direction of polarization is determined by the orientation of the individual neurons in the induced electric field (Ardolino et al. 2005).

1.3 Key tDCS variables, limitations and delivery parameters

There are several key variables that influence both the level and direction of the tDCS induced response. These include current density, stimulation session duration/timing and electrode polarity/positioning.

Current density determines the electrical field strength and is reliant on both the current strength and size of the electrodes (Nitsche et al. 2008). With respect to current strength, Nitsche and Paulus (2000) and Batsikadze et al. (2013) demonstrated that increasing motor cortex tDCS current intensity (whilst maintaining same electrode size) can result in prolonged, larger or reversed after effects on the level of motor pathway excitability in a healthy human population. These effects on motor pathway excitability can be elicited by simply measuring alterations to motor cortex TMS induced motor evoked potentials (MEP) amplitudes recorded both before and after tDCS (Nitsche, Paulus 2000).

With respect to electrode size, Nitsche et al. (2007) demonstrated that reducing stimulation electrode size area (whilst maintaining stimulation intensity) focused motor cortex tDCS induced after effects on motor pathway excitability in a healthy population. Using a 35cm^2 stimulation electrode size, so that the stimulation electrode area covered the motor cortex muscle representations for both the abductor digiti minimi and first dorsal interosseous muscles, tDCS induced similar excitability changes for the FDI and ADM representations. Reducing the stimulation electrode area to 3.5 cm^2 so that the stimulation electrode area only covered the motor cortex muscle representation for the ADM produced excitability changes for the ADM representation but not for the FDI.

Stimulation duration and timing are also key variables that can affect the tDCS induced response. Nitsche and Paulus (2000) demonstrated that increasing the length (current duration varied between 1 and 5 minutes) of 1mA motor cortex tDCS resulted in prolonged and larger tDCS after effects on the level of motor pathway excitability in a healthy population. Further, Monte-Silva et al. (2013) demonstrated that continuous application of 1mA motor cortex tDCS for 26 minutes resulted in motor pathway excitability enhancement whereas spaced stimulation of the same total duration (e.g. 2x13 min of tDCS with a 20 min interval) resulted in an abolishment or reduction of motor pathway excitability in a healthy population.

Current flow direction is another important variable that has been indicated to influence the tDCS induced response and depends mainly on the positions of the electrodes and their polarity. In conventional tDCS, both a positive (anode) and negative (cathode) electrode are utilised. The tDCS mechanism involves the movement of charged ions within the tissue (i.e. positive ions are attracted to a skin surface cathode; negative ions are attracted to a skin surface anode) (Schabrun 2010). With respect to electrode positioning, Nitsche and Paulus (2000) demonstrated that a motor cortex+contralateral forehead electrode arrangement achieved motor pathway excitability changes whereas motor-cortical, pre+post motor-cortical or occipital+contralateral forehead electrode arrangements could not in a healthy population.

With respect to electrode polarity, Nitsche and Paulus (2000) also demonstrated that cathodal motor cortex tDCS reduced motor pathway excitability whereas anodal motor cortex tDCS enhanced motor pathway excitability in a healthy population whilst maintaining stimulation duration and intensity.

It is also worth mentioning that there are limitations of the device. These include stimulation focality and stimulus intensity. Firstly, conventional tDCS does incorporate two electrodes (Nitsche et al. 2007). Hence, the current flows throughout the brain between the two electrodes potentially producing nerve polarization over a large area of the brain (Priori, Hallett & Rothwell 2009).

Alternative montages, such as the 4 x 1 high definition electrode montage, have and are still being explored. Borckardt et al. (2012) has shown, using magnetic resonance imaging (MRI) derived computational models, that high definition electrode montages produce a more restricted current flow with comparable efficacy to conventional tDCS.

Another key tDCS limitation is stimulus intensity. TMS applied over the motor cortex of one hemisphere at an adequate intensity can elicit a motor response (i.e. twitch) in the targeted muscle on the contralateral side. This motor response therefore represents an active biological marker or phemenological indicator of stimulation success (Priori, Hallet & Rothwell, 2009). In contrast, there are no standard protocols/markers to assess effectiveness of tDCS strength (or dose) (Priori, Hallet & Rothwell, 2009). Unlike TMS, tDCS strength is therefore not individually adjusted.

There also exist recommended delivery parameters, which relate to the length and current density of stimulation. Conventional tDCS has repeatedly been shown to be delivered safely in humans when applied with current strengths ≤ 2mA, through electrodes ~25-35cm^2 large for durations up to ~20 min per treatment session (Nitsche et al. 2003b, Iyer et al. 2005).

1.4 tDCS tolerability and safety aspects

Tolerability refers to the presence of uncomfortable and unintended effects (Woods et al. 2016). Headaches, local pain, dizziness, nausea, fatigue, skin redness and a tingling, itching or mild burning sensation under the area of the electrodes are known side effects with the use of tDCS (Brunoni et al. 2011). A phosphene sensation, associated with switching ‘on’ and ‘off’ the stimulation, has also been reported.

Safety aspects refer to damaging effects (Woods et al. 2016). Skin damage (i.e. burns) has previously been reported (Palm et al. 2008). tDCS induced structural brain damage is most unlikely and ruled out with certain protocols (Woods et al. 2016). tDCS induced structural brain damage in an in vivo rat model could only be achieved with a charge density 2 orders of magnitude higher than the charge density currently applied in humans (Liebetanz et al. 2009). Transient cognitive/behavioural disturbances have also been reported (Iuculano, Kadosh 2013).

There are procedures to help minimise side effects. For example, ramping (i.e. steadily changing) current intensity helps to reduce phosphene sensations. As well, preparing electrodes with saline solution and preventing direct contact between skin and electrodes helps to reduce skin irritation (Woods et al. 2016). Furthermore, adhering to safety guidelines and using thorough subject exclusion criteria further lower potential risks.

1.5 Clinical and research applications for tDCS

tDCS can be used either to modulate the steady state of the cortex by increasing or decreasing nervous system pathway excitability or to prime the nervous system to improve its responsiveness to other interventions (Schabrun 2010). In line with thesis related research goals, however, this chapter will not focus on research where tDCS has been used to prime the nervous system. This section will review research related to clinical and research applications for tDCS in the motor and sensory domains. It must be noted that the intention of this section is to provide a brief overview of research related to clinical and research applications for tDCS without critical appraisal. Critical appraisal of research most relevant to the thesis related research goals is provided with the Systematic Review in Chapter 2.

1.5.1 tDCS effects on the motor domain

Investigations examining the effects of tDCS on the motor domain have been carried out on both healthy and non-healthy populations. The effects of tDCS on two main outcome measures, namely motor pathway excitability and behavior, will now be explored in further detail. In line with this thesis research related goals, the following section also aims to highlight the effects of repeated stimulation sessions on abovementioned outcome measures.

1.5.1.1 tDCS induced changes to the excitability of the motor pathway in a healthy population

Research has investigated the effects of a single session of tDCS on the excitability of the motor pathway. As previously mentioned, motor cortex tDCS has been shown to alter the level of excitability of the motor pathway in a polarity-dependent manner in a healthy population (Nitsche, Paulus 2000).

Successive tDCS may also be able to influence tDCS induced changes to the level of excitability of the motor pathway. Alonzo et al. (2012) demonstrated that consecutive daily sessions of anodal motor cortex tDCS induced greater increases in MEP amplitude compared to second daily sessions of anodal motor cortex tDCS over a five day period. Interestingly, research later demonstrated that consecutive daily sessions of anodal motor cortex at a constant intensity (i.e. 2mA) or a gradually increasing intensity (i.e. 1-2mA) was equally effective in increasing motor pathway excitability in a healthy population (Gálvez et al. 2013). Furthermore, Bastani and Jaberzadeh (2014) demonstrated that with-in session repeated tDCS (i.e. two or three applications of 10 minute tDCS with an interval of 25 minutes) was preferable for modifying motor pathway excitability in a healthy population compared to a single application of 10 minute tDCS.

1.5.1.2 tDCS induced changes to behavioural measures of motor function

Motor cortex tDCS has been shown to influence aspects of motor behavior such as activities of daily living and motor learning in a healthy population. Boggio et al. (2006) and Hummel et al. (2010) demonstrated that a single session of anodal motor cortex tDCS could significantly improve Jebsen-Taylor Hand Function Test performance (i.e. time taken to perform activities of daily living with one hand) in both a young and older aged healthy human population but not after sham tDCS.

Alternatively, tDCS has also been shown to influence motor training. Reis et al. (2009) demonstrated that repeated daily sessions of anodal motor cortex tDCS in combination with motor training led to significantly enhanced motor

learning (the motor learning measure related to movement time and error rate of a sequential visual isometric pinch task) compared to sham tDCS. Analyses suggested that tDCS induced motor learning enhancement was primarily the result of a positive between sessions effect (i.e. offline consolidation). Interestingly, Reis et al. (2015) later demonstrated that tDCS applied only after the training of the sequential visual isometric pinch task did not induce skill gain.

Motor cortex tDCS has also been shown to influence motor function in different non-healthy populations such as Parkinson's disease and stroke. Fregni et al. (2006a) showed that a single session of anodal motor cortex tDCS significantly improved Unified Parkinson's Disease Rating Scale motor scores (motor score related to tremor, bradykinesia, rigidity, postural instability and gait) as well as simple reaction time scores (time taken to press a computer keyboard key using their index finger in response to computer presented stimuli) compared to sham tDCS in a Parkinson's disease population.

Alternatively, Fregni et al. (2005) demonstrated that a single session of cathodal tDCS delivered to the motor cortex of the affected hemisphere in stroke patients significantly improved Jebsen-Taylor Hand Function test performance but not with sham tDCS.

tDCS session frequency may also be able to influence tDCS induced changes to motor function. Boggio et al. (2007) later revealed that consecutive daily sessions of cathodal tDCS delivered to the unaffected hemisphere induced a greater percentage change from baseline in Jebsen-Taylor Hand Function

test performance compared to the Fregni et al. (2005) study findings (i.e. 16.7 % vs. 11.7 %). Hence, it can be seen that a number of papers have shed light relating to the potential clinical benefits of tDCS on motor function in different non-healthy populations.

1.5.2 tDCS effects on the sensory domain

Research examining the effects of tDCS on the sensory domain has also been carried out on both healthy and non-healthy populations. The effects of tDCS on the two main outcome measures of sensory pathway excitability and somatosensory perception will now be discussed in further detail. In line with research related goals, this section also aims to highlight the effects of successive tDCS on the aforementioned outcome measures.

1.5.2.1 tDCS induced changes to the excitability of the sensory pathway in a healthy population

Research has investigated the effects of a single session of tDCS on the excitability of the sensory pathway. A single session of either motor or sensory cortex tDCS has been shown to alter the level of excitability of the sensory pathway in a polarity-dependent manner (Dieckhöfer et al. 2006, Matsunaga et al. 2004). Changes to the level of sensory pathway excitability were probed by analysing peripheral nerve electrical stimulation induced somatosensory evoked potentials (SEP) recorded both before and after tDCS, with changes shown to outlast the stimulation period for up to one hour (Dieckhöfer et al. 2006). Alternatively, Sugawara et al. (2015) demonstrated that a single session of either motor and sensory cortex tDCS altered the level of excitability of the sensory pathway by measuring alterations to peripheral

nerve electrical stimulation induced somatosensory evoked magnetic fields (SEF) recorded both before and after tDCS.

1.5.2.2 tDCS induced changes to measures of somatosensory perception

Research has highlighted the ability of tDCS in altering somatosensory (i.e. discrimination/detection) and pain (i.e. detection/intensity) function in a healthy population. Psychophysical measures can give an indication of sensory and pain threshold changes while scales (i.e. numeric or visual analogue) can allow subjective rating of pain intensity. Rogalewski et al. (2004) showed that a single session of cathodal tDCS applied to the non-dominant motor cortex reduced tactile frequency discrimination thresholds for the non-dominant finger. No changes to tactile acuity, however, were seen with anodal and sham tDCS. Additionally, Boggio et al. (2008) showed that a single session of anodal motor cortex tDCS significantly increased current pain detection thresholds over time but not with sham tDCS. Research has also demonstrated that a single session of anodal motor cortex tDCS significantly reduced certain heat pain intensity scores compared to sham tDCS and/or natural history group (Aslaksen, Vasylenko & Fagerlund 2014).

Research has also investigated the potential clinical benefit of repeated sessions of tDCS on somatosensory processing abnormalities associated with disorders such as multiple sclerosis and persistent pain. Mori et al. (2012) demonstrated that 5 consecutive daily sessions of anodal somatosensory cortex tDCS improved spatial discrimination thresholds in multiple sclerosis patients as opposed to sham tDCS that showed no effect. Improvements lasted ~2 weeks post stimulation. Moreover, Fregni et al. (2006b)

demonstrated that 5 consecutive daily sessions of anodal motor cortex tDCS resulted in significantly greater pain improvement (i.e. pain measured using pain visual analogue score) compared to sham stimulation and stimulation of the pre-frontal cortex in patients with fibromyalgia.

1.6 Proposed molecular mechanisms for tDCS induced post-stimulation effects in humans

The mechanisms underpinning the after effects of tDCS are not completely understood. tDCS induced after-effects may involve alterations to the membrane potential, neurotransmitter (i.e. glutamate and gamma-amino-butyric acid {GABA}) involved synaptic transmission, protein level expression and neuromodulator activation (Nitsche et al. 2003a, Nitsche et al. 2004).

With respect to membrane potential changes, Nitsche et al. (2003a) showed that sodium and calcium channel blockers, namely carbamazepine and flunarazine, could effectively block motor cortex anodal tDCS induced post stimulus changes to MEP amplitudes. In line with a cathodal tDCS induced hyperpolarizing effect on the neuronal membrane, flunarazine and carbamazepine did not alter cathodal after effects.

With respect to neurotransmitter involved synaptic transmitter receptor changes, Nitsche et al. (2003a) further demonstrated that antagonizing glutamate receptors with the use dextromethorphane could abolish both anodal and cathodal motor cortex post stimulus effects on MEPs. Moreover, another study showed that lorazepam, a GABA agonist, enhanced and prolonged anodal tDCS induced effects on MEP amplitudes (Nitsche et al. 2004). Finally, Stagg et al. (2009) found that anodal motor cortex tDCS

significantly reduced magnetic resonance spectroscopy (MRS) detected GABA concentrations within the stimulated cortex, whilst cathodal motor cortex tDCS resulted in reduced MRS detected glutamate and GABA within the stimulated cortex.

With respect to protein level expression changes, research suggests that tDCS induced after effects may also be brain-derived neurotrophic factor (BDNF) dependent. Fritsch et al. (2010) demonstrated that combined tDCS and low frequency repeated electrical stimulation produced long-term potentiation (LTP) in motor cortex mouse slices. tDCS induced LTP, however, was absent in BDNF and tropomyosin related kinase B (TrkB) mutant mice. Another study also demonstrated using a retrospective analysis that carriers of the BDNF vall66met polymorphism, known to partially affect activity-dependent BDNF secretion, showed enhanced tDCS induced plasticity compared to Val66Val carriers (Antal et al. 2010).

Additionally, certain dopamine, adrenergic, acetylcholine and serotonin agents have previously been shown to alter anodal and cathodal tDCS induced after effects (i.e. abolish, enhance/prolong or turn inhibition into facilitation) (Nitsche et al. 2006, Nitsche et al. 2009, Nitsche et al. 2004, Kuo et al. 2007).

In summary, tDCS post stimulus effects may act via membrane, neuro-transmitter related synaptic transmission modulation, neuromodulator and neurotrophic based mechanisms. Further research appears to be required before firm conclusions can be made regarding the mechanisms of tDCS post stimulus effects.

1.7 Proposed central mechanisms for motor cortex tDCS induced post-stimulation effects on pain related outcome measures in humans

In line with the research goals of this thesis, it is worthwhile reviewing the proposed central and peripheral mechanisms for motor cortex tDCS induced post-stimulation effects on pain related outcome measures in humans. The proposed central mechanisms that will be discussed in further detail include influencing cortico-thalamic pathways, descending opioid-based anti-nociception and central stress related circuitry.

Firstly, motor cortex tDCS could alter pain related outcome measures by influencing descending cortico-thalamic pathways. Research has demonstrated using fMRI (i.e. seed functional coupling analysis) that motor cortex anodal tDCS could alter functional coupling between the ipsilateral motor cortex and thalamus in a healthy human population (Polanía, Paulus & Nitsche 2012). Lang et al. (2005) also demonstrated motor cortex tDCS induced changes to regional cerebral blood flow in the thalamus relative to sham tDCS in a healthy human population.

Motor cortex tDCS could also alter pain related outcome measures by influencing descending opioid-based anti-nociception. DosSantos et al. (2014) revealed that a single session of active and sham motor cortex tDCS affected Mu Opioid Receptor (MOR) activation differently in a healthy human population. Although both active and sham tDCS induced similar MOR activation in the precuneus and peri-aqueductal grey matter, only active tDCS induced MOR activation in the pre-frontal cortex whereas only sham tDCS induced MOR activation in the thalamus. Interestingly, only active tDCS

induced significant improvements to heat and cold pain thresholds compared to baseline and not after sham tDCS.

Finally, motor cortex tDCS could also alter pain related outcome measures by influencing stress related central nervous system circuitry. Cortisol is an end product of the hypothalamic-pituitary-adrenal (HPA) axis. Binkofski et al. (2011) demonstrated that a single session of anodal motor cortex tDCS could lower serum cortisol levels compared to sham tDCS in a healthy human population. The findings therefore suggest that motor cortex tDCS may be able to influence central stress related circuitry such as the hypothalamic-pituitary-adrenal (HPA) axis.

In summary, it is plausible to suggest that motor cortex tDCS analgesia in a healthy population could in part be attributable to influencing cortico-thalamic pathways, central opioid receptor activity or central stress related circuitry (Knotkova, Nitsche & Cruciani 2013).

1.8 Proposed peripheral mechanisms for motor cortex tDCS induced post-stimulation effects on pain related outcome measures in humans

The proposed peripheral mechanisms that will be discussed in further detail include influencing peripheral levels of nociceptive neuropeptides or stress related hormones.

The literature suggests a relationship between neuropeptides and nociception. Sensory neuropeptide substance P is involved in nociception and pro-inflammatory functions (Hernanz et al. 1993, Okano, Kuraishi & Satoh

1998) while research has also shown that levels of peripheral (i.e. plasma) substance P are significantly higher in chronic pain patients compared to healthy controls (Jang et al. 2011).

Research also suggests a relationship between stress related hormones and nociception. Cortisol is an end product of the hypothalamic-pituitary-adrenal (HPA) axis. Persistent pain states may result in abnormal HPA stimulation, which for a certain amount of time can result in exaggerated levels of serum cortisol (Tennant, Hermann 2002). Research has also shown that salivary concentrations of cortisol are significantly higher in chronic pain patients compared to healthy controls (Vachon-Presseau et al. 2013).

Hence, it could be postulated that tDCS induced effects on nociception could be associated with changes in the level of nociceptive peripheral neuropeptides such as substance P or stress related hormones such as cortisol.

Chapter 2

Systematic Review

2.1 Introduction / Aim

Reviews of the literature revealed that there are some investigations that have demonstrated that repeated daily sessions of tDCS could induce lasting effects in the motor domain in a healthy population and both the sensory and motor domains in a clinical population (Boggio et al. 2007, Mori et al. 2012, Reis et al. 2009). Of interest is whether increasing stimulation session frequency can evoke cumulative and lasting body sensory system function alteration in a healthy population. The aim of this review was to perform a systematic evaluation of the literature on research specific to the application of tDCS to a healthy human population in two distinct domains:

1) the effects of sensory cortex tDCS on sensory threshold related outcome measures

2) the effects of motor cortex tDCS on pain threshold and pain intensity related outcome measures.

2.2 Methods

2.2.1 Systematic review design

The PRISMA guidelines for systematic review reporting were utilised as guidelines for conducting this systematic review (Moher et al. 2009).

2.2.2 Search strategy

A search of 10 electronic databases including PubMed, CINAHL, EMBASE, PEDro, Informit Health Collection, Ovid Medline, Scopus, ACP Journal Club, The Cochrane Library, and PsychINFO was conducted in Jan 2015 to locate potential articles. The search terms included: transcranial direct current stimulation, Transcranial Direct Current Stimulation, tDCS, pain, analgesia, healthy, qst, sensor*, percept*, psychophysic* and sensation. The search strategy included the Boolean operators ‘and’ and ‘or’ in order to combine the search terms (refer to Table 1). The reference lists of the included studies were additionally searched to ensure all relevant articles would be identified.

Table 1 Database strategy and results

Database	Search strategy	Result
Embase	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	199
PubMed	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	119
PEDro	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	0
CINAHL	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	14
Scopus	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	135
Informit Health Collection	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	0
Ovid Medline	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	97
ACP Journal Club	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	0
PsychINFO	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	75
Cochrane Library	((("transcranial direct current stimulation" OR "Transcranial Direct Current Stimulation" OR tdcs)) AND ((sensor* OR "Sensation" OR percept* OR qst OR psychophysic*) OR (pain OR "Pain" OR analgesia)) AND (healthy))	27

2.2.3 Eligibility criteria

2.2.3.1 Rationale for inclusion/exclusion criteria

Search specific inclusion/exclusion criteria were used to produce 2 results, so that the two distinct aforementioned domains of the literature were reviewed (refer to Table 2). The first set of search results (Review A) includes literature that has investigated the effects of sensory cortex tDCS on sensory threshold related outcome measures in a healthy human population. The second set of results (Review B) includes literature that has investigated the effects of motor cortex tDCS on pain threshold and pain intensity related outcome measures in a healthy human population.

2.2.3.2 Screening and selection procedures

Initial screening of titles and abstracts in line with study specific inclusion and exclusion criteria took place to isolate potential relevant articles. Screening of extracted full text papers in line with study specific inclusion and exclusion criteria was then performed for final study eligibility and then divided into Review A and Review B (refer to Figure 1). The primary investigator screened all titles, abstracts, and full text papers prior to making a decision about study eligibility.

Table 2 Inclusion and exclusion criteria

Criteria Category	Inclusion	Exclusion
Review A		
Participants	• Mean or lower limit of range ≥ 18 yrs • Healthy human	• Less than 18 years of age • Non healthy human
Intervention	• Anodal or cathodal tDCS delivered to the sensory cortex.	• tDCS only at locations other than sensory cortex. • Non-conventional forms of tDCS (e.g. high definition-tDCS) only. • tDCS only is not an intervention (e.g. tDCS and a drug).
Comparison	• sham-tDCS	• Does not have sham-tDCS
Outcomes	• Sensory threshold for electric, mechanical, thermal or laser modalities	• Does not have sensory threshold for electric, mechanical, thermal or laser modalities
Trial design	• Parallel, controlled • Cross-over, controlled	• Review article • Selective review
Type of publication	• Peer-reviewed journal articles • Written in English	• Non peer-reviewed journal article • Not written in English
Review B		
Participants	• Mean or lower limit of range ≥ 18 yrs • Healthy human	• Less than 18 years of age • Non healthy human
Intervention	• Anodal or cathodal tDCS delivered to the motor cortex.	• tDCS only at locations other than motor cortex. • Non-conventional forms of tDCS (e.g. high definition-tDCS) only. • tDCS only is not an intervention (e.g. tDCS and a drug).
Comparison	• sham-tDCS	• Does not have sham-tDCS
Outcomes	• Pain threshold/intensity for electric, mechanical, thermal or laser modalities	• Does not have pain threshold/intensity for electric, mechanical, thermal or laser modalities
Trial design	• Parallel, controlled • Cross-over, controlled	• Review article • Selective review
Type of publication	• Peer-reviewed journal articles • Written in English	• Non peer-reviewed journal article • Not written in English

2.2.3.3 Data extraction procedures

The data extracted from the studies relevant to the aims of this overall review were separated into 1) Methods (i.e. study design, sample size, body stimulation method, related outcome measure, Table 4) and delivery of intervention (i.e. tDCS type, stimulating electrode size, current intensity, current density, stimulation duration, Table 5) and 2) Results (i.e. sensory and pain outcome measure changes, Tables 6 and 7). Data was also extracted from two recent related systematic reviews to allow comparison (Vaseghi, Zoghi & Jaberzadeh 2014, Vaseghi, Zoghi & Jaberzadeh 2015b).

2.2.4 Methodological quality assessment

The modified Downs and Black 27 item checklist was used to evaluate the quality of included studies in this review (Table 3) (Downs, Black 1998). The areas the checklist assesses include: study reporting; external validity; internal validity; bias/confounding, and statistical power (Downs, Black 1998). The checklist has been evaluated as a suitable quality assessment tool for randomised and non-randomised intervention studies (Saunders et al. 2003). Two reviewers independently scored all papers. If there was a disagreement, consensus was gained by discussion. As in previous studies, the tool was modified for use in this review (i.e. the question concerning statistical power was scored 1 if a power or sample size calculation was present and 0 if no power or sample size calculation was present) (Chudyk et al. 2009). Therefore, the total score was out of 28. The Downs and Black scores can be separated into 4 quality categories: excellent (26 to 28), good (20 to 25), fair (15 to 19), and poor (≤14) (Hooper et al. 2008).

2.2.5 Quantifying tDCS induced changes

The percentage change from baseline value was obtained using exact percentage change from baseline values, exact mean values or graphed mean values (i.e. estimated; indicated by a ~) provided for at least one relevant outcome measure. A percentage change from baseline value assessment has previously been performed in a recent systematic review (Vaseghi, Zoghi & Jaberzadeh 2014). The obtained percentage change from baseline values for sensory, pain and pain intensity related outcome measures are outlined in Tables 6 and 7. Two included studies were excluded from this analysis because the percentage change from baseline value for any relevant outcome measure could not be calculated. No attempt was made to pool data, as the reviewers deemed combining the diverse outcome measures reported nonsensical.

2.2.6 Analysis of results

After establishing study eligibility, the single student investigator then used a critical narrative synthesis approach in order to critique, compare and contrast the results from the included studies.

2.3. Results

2.3.1 Search Strategy

Figure 1 presents a PRISMA flow diagram. Electronic database and reference list searches yielded 1375 potential articles. After abstract and title

review and removal of duplicates, 16 articles were retrieved in full text. After full text review, 14 articles met the inclusion criteria.

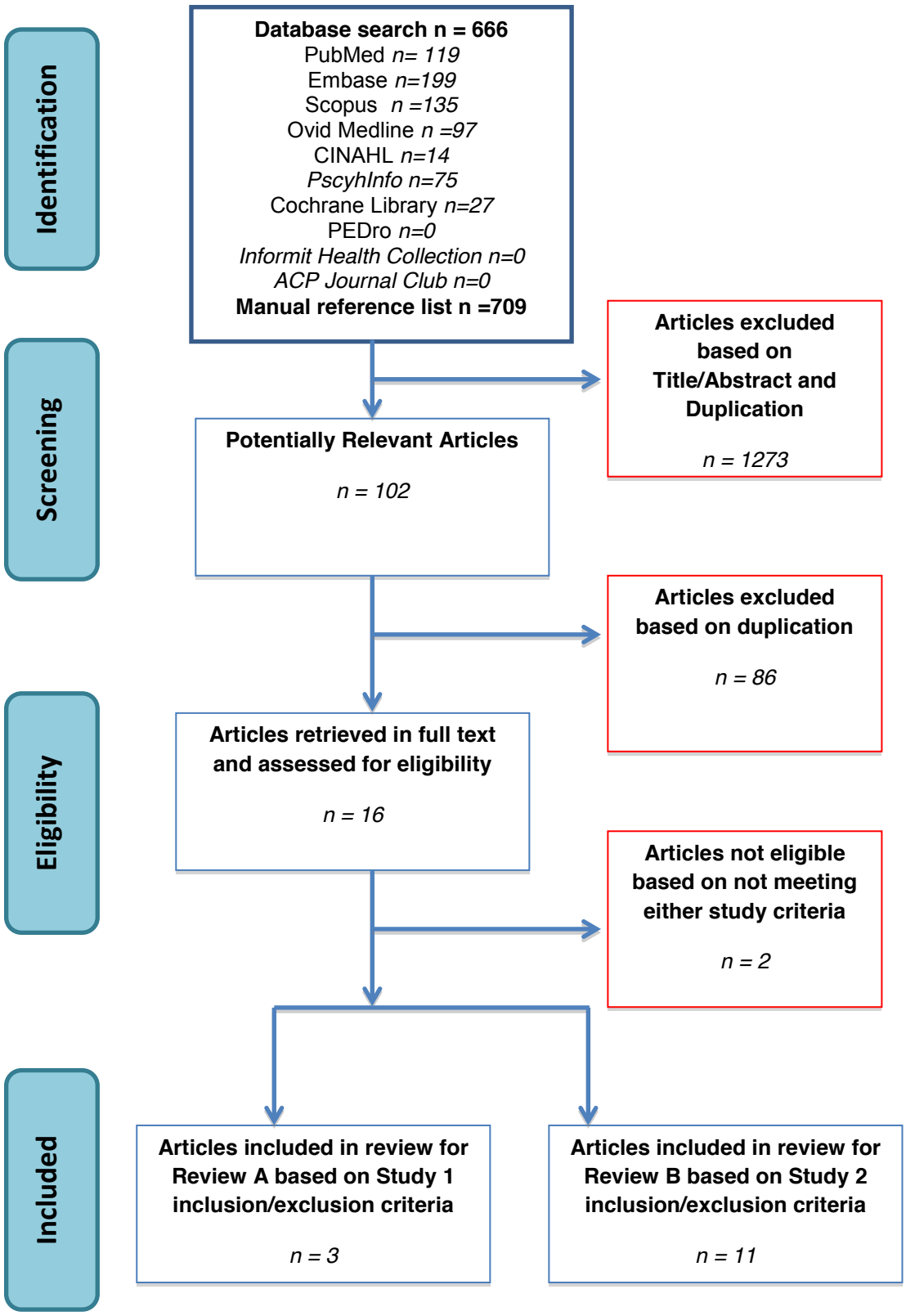

Figure 1 Prisma flow diagram for systematic review

2.3.2 Methodological quality assessment

Table 3 demonstrates the Downs and Black scores and quality category for included studies. The range of scores within Review A was 22-24. All 3 articles were of a good quality of evidence. While the range of scores was 18-23 for Review B with 2 articles fair in quality of evidence and 9 articles good in quality of evidence. Most studies did not provide a comprehensive attempt to measure potential important adverse effects (item 8), the source population for participants (Item 11), the research setting (Item 13) and sample size power calculations (item 27).

Table 3 Breakdown of Downs and Black (D&B) scoring

Paper	Reporting										External Validity			Internal Validity - Bias							Internal Validity - Confounding						Power	D&B score	D&B category
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Out of 28	
Review A																													
(Ragert et al. 2008)	1	1	1	1	2	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	24	Good
(Fugimoto et al. 2014)	1	1	1	1	2	1	1	0	1	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0	22	Good
(Grundmann et al. 2011)	1	1	1	1	2	1	1	0	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	22	Good

Paper	Reporting										External Validity			Internal Validity - Bias							Internal Validity - Confounding						Power	D&B score	D&B category
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Out of 28	Good
Review B																													
(Hansen et al. 2011)	1	1	1	1	2	1	1	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	23	Good
(Bachmann et al. 2010)	1	1	1	1	2	1	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	20	Good
(Boggio et al. 2008)	1	1	1	1	2	1	1	0	0	1	0	0	0	1	1	1	1	1	0	1	1	0	1	1	1	1	0	20	Good
(Reidler et al. 2012)	1	1	1	1	2	0	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	23	Good
(Hamner et al. 2014)	1	1	1	1	2	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	21	Good
(Aslaksen, Vasylenko & Fagerlund, 2014)	1	1	1	1	2	1	1	0	1	1	0	1	0	1	1	1	0	1	1	0	1	0	1	1	1	1	0	21	Good
(DosSantos et al. 2014)	1	1	1	1	2	1	0	0	1	1	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	1	0	19	Fair
(Ihle et al. 2014)	1	1	1	1	2	0	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	22	Good
(Zandieh et al. 2013)	0	1	1	1	2	1	0	0	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	18	Fair
(Csifcsak et al. 2009)	1	1	1	1	2	1	1	0	1	1	0	1	0	1	0	1	1	0	1	0	1	1	1	0	1	1	0	20	Good
(Jurgens et al. 2012)	1	1	1	1	2	1	0	0	0	1	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	20	Good

2.3.3 Methodological parameters

2.3.3.1 Study design

Study design characteristics for Review A and B are outlined in Table 4. The 3 studies in Review A were all crossover designed while 10 of the 11 studies in Review B were crossover designed. The remaining 1 study was a parallel design.

2.3.3.2 Participant numbers

Participant number characteristics for included studies are outlined in Table 4. The total number of participants across the studies in Review A was 31 with the number of participants ranging from 9 to 12. The total number of participants across the studies was 225 in Review B with the number of participants ranging from 8 to 75.

2.3.3.3 Outcome measures

Thesis related outcome measure and body stimuli types found in included studies are outlined in Table 4. The outcome measure types used by studies in Review A and B included sensory and pain thresholds as well as pain intensity scores. Body stimuli type included thermal and mechanical for studies in review A. The list of body stimuli type found in studies in review B included electrical, thermal, mechanical and laser.

2.3.3.4 tDCS interventions

tDCS intervention characteristics for included studies are outlined in Table 5. The stimulating electrode size, stimulation intensity, current density and stimulation duration / stimulation session across the studies in Review A was 25 to 35 cm^2, 1mA, 0.029 to 0.04 mA / cm^2 and 15 to 20 minutes respectively. The stimulating electrode size, stimulation intensity, current density and

stimulation duration / stimulation session across the studies in Review B was 16 to 35 cm^2, 1 to 2 mA, 0.029 to 0.063 mA / cm^2 and 5 to 40 minutes respectively. The type of direct current stimulation found in studies for both Review A and B included anodal and cathodal.

Table 4 Included study characteristics

Paper	Trial design (s-tDCS = sham tDCS)	Sample size	Stimulation method (for outcome measure)	Related outcome measures
Review A				
(Grundmann et.al. 2011)	s-tDCS, cross-over, single blinded	12	Mechanical & Thermal	Sensory & Pain Threshold; Pain Intensity (numeric rating scale)
(Fujimoto et al. 2014)	s-tDCS, cross-over, double blinded	9	Mechanical	Sensory Threshold
(Ragert et al. 2008)	s-tDCS, cross-over, double blinded	10	Mechanical	Sensory Threshold
Review B				
(Boggio et al. 2008)	s-tDCS, cross-over, double blinded	20	Electric	Sensory & Pain Threshold
(Hansen et al. 2011)	s-tDCS, cross-over, single blinded	19	Electric	Pain Threshold & Pain Intensity (numeric rating scale)
(Bachmann et al. 2010)	s-tDCS, cross-over, single blinded	8	Mechanical & Thermal	Sensory & Pain Threshold; Pain Intensity (numeric rating scale)
(Reidler et al. 2012)	s-tDCS, cross-over, double blinded	15	Mechanical & Thermal	Sensory & Pain Threshold
(Jurgens et al. 2012)	s-tDCS, cross-over, single blinded	17	Mechanical & Thermal	Sensory & Pain Threshold; Pain Intensity (visual analogue scale)
(Csifcsak et al. 2009)	s-tDCS, cross-over single blinded	10	Laser	Sensory & Pain Threshold

(Hamner et al. 2014)	s-tDCS, cross-over, single blinded	15	Thermal	Pain Intensity (visual analogue scale)
(Zandieh et al. 2013)	s-tDCS, cross-over, unable to determine blinding	22	Thermal	Sensory & Pain Threshold; Pain Intensity (numeric rating scale)
(Ihle et al. 2014)	s-tDCS, cross-over, double blinded	15	Mechanical & Thermal	Pain Threshold & Pain Intensity (visual analogue scale; numeric rating scale)
(Aslaksen, Vasylenko & Fagerlund 2014)	s-tDCS, parallel, double blinded	75	Thermal	Pain Threshold & Pain Intensity (visual analogue scale)
(DosSantos et al. 2014)	s-tDCS, cross-over, single blinded	9	Thermal	Pain Threshold

Table 5 tDCS parameters of included studies

Paper	Type of tDCS a = anodal c = cathodal	Electrode size (cm^2)	Intensity (mA)	Current density (mA/cm^2)	Time of stimulation (min)
Review A					
(Fujimoto et al. 2014)	a + c-tDCS	25	1	.04	20
(Ragert et al. 2008)	a-tDCS	25	1	.04	20
(Grundmann et al. 2011)	a + c-tDCS	35	1	.029	15
Review B					
(Bachmann et al. 2010)	a + c-tDCS	35	1	.029	15
(Boggio et al. 2008)	a-tDCS	35	2	.057	5
(Reidler et al. 2012)	a-tDCS	35	2	.057	20
(Csifcsak et al. 2009)	a + c-tDCS	35	1	.029	10
(Hansen et al. 2011)	a + c-tDCS	16	1	.063	20
(Hamner et al. 2014)	a-tDCS	35	2	.057	40
(Zandieh et al. 2013)	a + c-tDCS	35	2	.057	15
(Ihle et al. 2014)	a + c-tDCS	35	1	.029	15
(Jürgens et al. 2012)	a + c-tDCS	35	1	.029	15
(Aslaksen, Vasylenko & Fagerlund 2014)	a-tDCS	35	2	.057	7
(DosSantos et al. 2014)	a-tDCS	35	2	.057	20

Presentation of certain results (i.e. relating to quantifying tDCS induced change) and relevant discussion (i.e. relating to methodological quality, tDCS methods and quantifying tDCS induced change) for reviews A and B has been separated for ease of reading.

2.3.4 Quantifying tDCS induced changes – Review A

Percentage change from baseline values found in included studies are outlined in Table 6. The range of percentage changes from baseline for sensory thresholds following anodal, cathodal and sham tDCS respectively is listed below:

- Sensory thresholds:
    - Anodal; -29.3 to +0.8
    - Cathodal: +1.7 to +2.5
    - Sham; -0.3 to +1.6

Table 6 Review A percentage changes compared to pre-tDCS

Paper	Stimulus type (for outcome measure)	Location (for outcome measure)	C or I †	Estimated from graph (~)	Mean % change following anodal tDCS *	Mean % change following cathodal tDCS *	Mean % change following sham tDCS *
(Fugimoto et al. 2014)	Mechanical (i.e. grating orientation)	finger	C		-10		+1.6
(Ragert et al. 2008)	Mechanical (i.e. grating orientation)	finger	C		-29.3		0
(Grundmann et al. 2011)	Thermal (i.e. cold)	hand	C	~	+0.8	+2.5	-0.3
			I	~	+0.8	+1.7	-0.1

† C = contralateral body side to tDCS; I = ipsilateral body side to tDCS

* + = increase; - = decrease

2.3.5 Discussion – Main findings for Review A

Following our systematic literature review 3 studies, meeting inclusion criteria, were identified to provide a summary of the literature that has investigated the effects of sensory cortex transcranial direct current stimulation on body sensory threshold related outcome measures in a healthy population.

With respect to methodological quality, overall the 3 studies demonstrated a good-quality mean method score of 22.66/28 with scores ranging from 22-24 (refer to Table 3).

Of interest, most studies lacked information relating to potential adverse effects (item 8), participant source (item 11), research setting (item 13) and statistical power reporting (item 27).

When cross-referencing the scored studies for two recent and our systematic review only one paper (Grundmann et al. 2011) was evaluated using the Downs and Black scoring method. This occurred as it appears that neither Antal et al. (2008) nor Rogalewski et al. (2004) were included in our systematic review due to a variation in specific inclusion and exclusion criteria. Furthermore, Vaseghi, Zoghi & Jaberzadeh (2014) only included studies published up to July 2012.

Of particular note was that the methodological scoring carried out in this review for Grundmann et al. (2011) was 6 points higher compared to scoring reported in two similar recent systematic reviews (Vaseghi, Zoghi & Jaberzadeh 2014, Vaseghi, Zoghi & Jaberzadeh 2015b).

It is difficult to exactly establish the reason for this difference as the two previous systematic reviews do not provide a breakdown of the Downs and Black thus not allowing a scoring comparison to be carried out.

Our higher Downs and Black checklist score could be attributed to differences in how items related to study design and participant flow information were interpreted and scored. Of note were that all included studies for review A were a crossover designed. As a result, the scorers automatically scored items 21, 23 and 25 a 1 and item 5 a score of 2. Further, although studies did not present detailed participant flow information (e.g. numbers of participants enrolled versus analysed), the scorers automatically scored items 9, 19 and 26 a score of 1 if participant numbers appeared to be constant throughout the entire manuscript.

With respect to methods, the sensory cortex tDCS variables used were fairly consistent (refer to Table 5). It is also worthy to note that none of the included studies investigated the use of repeated daily tDCS. Secondly, the sample sizes were small (refer to Table 4), which is in line with a lack of sample size power calculation information. Finally, the study designs were relatively consistent and all studies appeared to use mechanical modality sensory thresholds.

With respect to quantifying tDCS induced change, percentage change from baseline sensory threshold values following anodal, cathodal or sham sensory cortex tDCS overall ranged from -29.3 to +2.5 (refer to Table 6). However, 9/10 values were ≤ 10 %.

When cross-referencing the studies that had a percentage change from baseline score reported for the two recent and one present systematic review, it appeared that only Grundmann et al. (2011) were scored by all three systematic reviews. As well, only Vaseghi, Zoghi and Jaberzadeh (2014) and the present systematic review provide a percentage change from baseline score for Ragert et al. (2008).

With respect to percentage change from baseline findings calculated for Grundmann et al. (2011) and Ragert et al. (2008) these were higher than those reported by our systematic review.

Data extraction differences may help to explain this. Firstly, Grundmann et al. (2011) report tDCS induce changes to cold detection thresholds as the difference detected against a baseline temperature of 32 degrees celsius. Hence, a mean value change from -1.2 degrees celsius to -2.1 degrees celsius (difference from baseline; 32 degree celsius) would correspond to ~ 75% change from baseline. In contrast, using the current systematic review methodology, a mean value change from 30.8 degrees celsius to 20.9 degrees celsius would correspond to ~3% change from baseline. In addition, the present systematic review did not contact authors for means of desired outcome measures when means were not presented numerically or accessible from figures and graphs. As a result, the review did also not report the Grundmann et al. (2011) heat, mechanical and vibration sensory threshold percentage change from baseline scores.

The slight variability present in percentage change from baseline values may be due to outcome measure type. Greater percentage change from baseline

values were reported for effects of anodal sensory cortex tDCS on mechanical sensory discrimination thresholds compared to thermal sensory detection thresholds (refer to Table 6).

2.3.6 Quantifying tDCS induced changes – Review B

Percentage change from baseline values found in the included studies are outlined in Table 7. The range of percentage changes from baseline for each outcome measure type following anodal, cathodal and sham tDCS respectively is listed below:

- Sensory thresholds:
    - Anodal; -10 to +10.7
    - Cathodal: -3.4 to +96.2
    - Sham; -0.3 to +9.7

- Pain thresholds:
    - Anodal; -2.9 to +85
    - Cathodal: 0.2 to +5.9
    - Sham; -0.8 to +75

- Pain intensity:
    - Anodal; -40.9 to +16.2
    - Cathodal: -2.2 to +5.88
    - Sham; -37.5 to +31

Table 7 Review B Percentage changes compared to pre-tDCS

Paper	Stimulus type (for outcome measure)	Location (for outcome measure)	C or I †	Estimated from graph (~)	Mean % change following anodal tDCS *	Mean % change following cathodal tDCS *	Mean % change following sham tDCS *
			Sensory threshold changes				
(Boggio et al. 2008)	Electric	finger	C		+6.5		-0.3
(Csifcsak et al. 2009)	Laser (i.e. warm)	hand	C	~	-10	-3.4	+1.3
(Bachmann et al. 2010)	Thermal (i.e. cold)	hand	C	~	+0.3	+2.0	+0.5
			I	~	+1.3	+1.2	+1.0
	Mechanical (i.e. von Frey-	hand	C	~	-3.0	+96.2	+9.7
			I	~	+10.7	+16.7	+7.7
			Pain threshold changes				
(Aslaksen, Vasylenko & Fagerlund 2014)	Thermal (i.e. heat)	forearm	C		+1.7		+2.3
(Boggio et al. 2008)	Electric	hand	C		+8.3		+0.4
(Ihle et al. 2014)	Thermal (i.e. heat)	hand	C	~	-1.1	+0.2	-0.5
(DosSantos et al. 2014)	Thermal (i.e. heat)	face	I	~	+2.3		-0.4
			C	~	+2.1		-0.8
	Thermal (i.e. cold)	face	I	~	+84.4		+40.6
			C	~	+85		+75
(Csifcsak et al. 2009)	Laser	hand	C	~	-2.9	+5.9	-0.8

					Pain intensity changes		
(Aslaksen, Vasylenko & Fagerlund 2014)	Thermal (i.e. heat)	forearm					
	43°C		C		-25.2		-20.0
	45°C		C		-36.7		-22.6
	47°C		C		-21.9		-9.4
(Jurgens et al. 2012)	Thermal (i.e. heat)	forearm	C		+9.38	+5.88	+31.0
(Hansen et al. 2011)	Electrical	finger	C		+16.2	0	0
		head	C		+4.7	-2.2	0
(Hamner et al. 2014)	Thermal (i.e. cold)	forearm					
	14°C		C	~	-40.9		-37.5
	7°C		C	~	-19.1		-16.3
	0°C		C	~	+1.5		-3.0

† C = contralateral body side to tDCS; I = ipsilateral body side to tDCS

* + = increase; - = decrease

2.3.7 Discussion – Main findings for Review B

Eleven studies that met the inclusion criteria were identified to evaluate the literature that has investigated the effects of motor cortex transcranial direct current stimulation on pain threshold and pain intensity related outcome measures in a healthy population.

With respect to methodological quality, overall the 11 papers demonstrated a good-quality mean method score of 20.64/28 with scores ranging from 18-23 (refer to Table 3).

Of interest, most studies lacked information relating to potential adverse effects (item 8), participant source (item 11), research setting (item 13) and statistical power reporting (item 27). As well, only roughly half of the studies performed double blinding (items 15 and 24). Interestingly, only 2 papers measured effectiveness of blinding with sham tDCS (Ihle et al. 2014, Reidler et al. 2012).

Studies that were scored by our present systematic review and at least 1 of the systematic reviews published by Vaseghi, Zoghi and Jaberzadeh in 2014 and 2015 include only Csifisak et al. (2009), Hansen et al. (2011) and Boggio et al. (2008).

There are reasons for differences in the variance of the studies included or not included in the three different reviews. Firstly, the present systematic review did not include Terney et al. (2008) due to review specific inclusion and exclusion criteria. As well, only studies published to July 2012 were included in Vaseghi, Zoghi and Jaberzadeh (2014).

The methodological scoring carried out in this review was 2-6 points higher compared to scoring reported in the two past systematic reviews. Reasoning for methodological quality scoring differences is outlined in the discussion for Review A.

With respect to methods, relatively similar tDCS variables were used (refer to Table 5). Interestingly, repeated daily tDCS was not used in any included studies. Secondly, included studies generally had relatively small sample sizes (refer to Table 4). Additionally, the study designs were fairly similar. Finally, most studies used thermal modality sensory thresholds.

With respect to quantifying tDCS induced change, the results will be discussed in relation to 3 distinct areas of sensory thresholds, pain thresholds and pain intensity.

Firstly, percentage change from baseline sensory threshold values following anodal, cathodal or sham motor cortex tDCS ranged from -10 to +96.2 (refer to Table 7). However, 14/17 values were ≤ 10%.

Studies that were percentage change from baseline sensory threshold scored by our present systematic review and at least 1 of the systematic reviews published by Vaseghi, Zoghi and Jaberzadeh in 2014 and 2015 include only Csifisak et al. (2009), Bachmann et al. (2010) and Boggio et al. (2008).

Of note, the percentage change from baseline scores for sensory thresholds calculated for Csifisak et al. (2009), Bachmann et al. (2010) and Boggio et al. (2008) in the present systematic review were generally smaller to those reported in the 2 recent systematic reviews (Vaseghi, Zoghi & Jaberzadeh

2014, Vaseghi, Zoghi & Jaberzadeh 2015b). Refer to the discussion for review A for reasoning for percentage change scoring differences.

The variability in motor cortex induced sensory threshold percentage change from baseline values may be due to outcome measure type. For example, the biggest percentage change from baseline values in this review was reported for effects of cathodal tDCS on mechanical (i.e. Von Frey) sensory thresholds (i.e. + 96.2). In contrast, percentage change from baseline values in this review for effects of tDCS on thermal (i.e. cold), electric and laser pain thresholds measure were all less than 10 percent.

Secondly, percentage change from baseline pain threshold values following anodal, cathodal or sham motor cortex tDCS ranged from -2.9 to +85 (refer to Table 7). However, 14/18 values were ≤ 10%.

Studies that were percentage change from baseline pain threshold scored by our present systematic review and at least 1 of the systematic reviews published by Vaseghi, Zoghi and Jaberzadeh in 2014 and 2015 include only Csifisak et al. (2009) and Boggio et al. (2008).

Of note, the percentage change from baseline scores for pain thresholds in the present systematic review for Csifisak et al. (2009) and Boggio et al. (2008) were typically smaller than those reported in recent systematic reviews.

One reason for this may be due to possible incorrect data extraction. For example, Boggio et al. (2008) specifically provide percentage change from

baseline values that do not line up with those reported in Vaseghi, Zoghi and Jaberzadeh (2014).

The variability in motor cortex induced pain threshold percentage change from baseline values may be due to outcome measure type. For example, the biggest percentage change from baseline values in this review was reported for effects of tDCS on face thermal (i.e. cold) pain thresholds (i.e. +85). In contrast, percentage change from baseline values in this review for effects of tDCS on thermal (i.e. heat), electric and laser pain thresholds measure were all less than 10 percent.

Thirdly, percentage change from baseline values following anodal, cathodal or sham motor cortex tDCS for pain intensity scores ranged from -40.9 to +31 (refer to Table 7).

Interestingly, both the recent Vaseghi systematic reviews did not extract percentage change from baseline scores for pain intensity related outcome measures in a healthy population (Vaseghi, Zoghi & Jaberzadeh 2014, Vaseghi, Zoghi & Jaberzadeh 2015b).

It is also important to note that motor cortex tDCS induced pain intensity percentage change from baseline values may be affected by outcome measure type and body location. For example, bigger percentage change from baseline values in this review were reported for effects of anodal tDCS on forearm thermal pain intensity scores compared to effects of tDCS on finger/head electrical pain intensity scores.

2.3.8 What's new

Compared to the two previous systematic reviews published by Vaseghi, Zoghi and Jaberzadeh in 2014 and 2015, our systematic review involved the inclusion of 6 new trials.

Methodological differences (i.e. differences in quality scoring, data extraction and included studies) have presumably led to altered conclusions regarding methodological quality and tDCS induced change.

With respect to methodological quality, the analysis suggests that overall methodological quality was good.

With respect to quantifying active tDCS induced changes compared to baseline, the analysis suggests that overall percentage changes from baseline were variable but mostly minimal (i.e. ≤ 10 %).

Finally, our current systematic review quantified tDCS induced changes on pain intensity compared to baseline in a healthy population. tDCS induced changes on pain intensity were generally slightly greater compared to tDCS induced changes on sensory and pain thresholds.

2.3.9 Strengths and limitations of the review

The review is subject to certain limitations. To begin with, some of the mean values obtained for quantifying tDCS induced changes had to be retrieved from graphs from the papers. This methodology may therefore have introduced data inaccuracy. The review was also limited to English-language articles.

2.3.10 Conclusions and research implications

In summary, there have been numerous investigations into the effects of sensory and motor cortex transcranial direct current stimulation on body sensory and pain related outcome measures in a healthy population. Critical appraisal of this literature revealed key methodological quality limitations, which have impaired the quality of evidence. Critical appraisal of the literature also revealed that single session anodal, cathodal and sham motor and sensory cortex tDCS induced inconsistent but mostly minimal percentage change from baseline values for body sensory and pain related outcome measures. Future efforts may therefore benefit by increasing stimulation frequency (e.g. using repeated daily tDCS), which might help to establish more consistent effects on physical sensory/pain thresholds (i.e. detection/ tolerance) and pain intensity in a healthy population.

The following section provides the framework for a comprehensive project investigating the effects of repeated sessions of tDCS on somatosensory function in a healthy population and the validity for assessing pain with physical quantitative sensory measures (psychophysical devices) compared to the subjective pain measures (i.e. Visual Analogue Scale (VAS); self-reported general pain sensitivity questionnaire (SRGPSQ)) and objective pain related salivary biomarkers.

2.4 Study design rational and significance

Numerous studies have investigated the effects of transcranial direct current stimulation (tDCS) on measures of somatosensory perception in a healthy

population. However, systematic reviews indicate both methodological limitations and heterogenous tDCS induced effects for existing trials. The reviews also reveal that stimulation frequency (e.g. using repeated daily tDCS) is one area that researchers have failed so far to focus their attempts on.

The objective of the first study (that follows) was therefore to investigate the effects of repeated daily sessions of tDCS on psychophysical measures of somatosensory function only by measuring the effects of repeated daily tDCS on vibration detection thresholds in a healthy human population.

The second study had three distinct objectives. The primary objective was to investigate the effects of repeated daily sessions of tDCS on psychophysical and subjective measures of somatosensory function (i.e. detection and perception) by measuring the effects of repeated daily tDCS on psychophysical thresholds (i.e. electric, thermal and mechanical pressure detection and pain thresholds) and subjective pain scores (i.e. electric, thermal and mechanical pain visual analogue scales) in a healthy human population.

The second objective was to investigate the effects of repeated daily sessions of tDCS on objective pain related biological markers by measuring the effects of repeated sessions of tDCS on salivary cortisol and substance P levels post experimental pain stimulation in a healthy human population.

The third and final objective of the second study was to investigate the associations and/or differences between different psychophysical thresholds (i.e. electrical, mechanical and thermal detection and pain thresholds),

subjective pain measures (i.e. electrical, mechanical and thermal visual analogue scales (VAS); self-reported general pain sensitivity questionnaire (SRGPSQ)) and objective pain related biomarkers (i.e. salivary substance P and cortisol levels) in a healthy human population.

Measuring tDCS induced effects on psychophysical, subjective and objective outcome measures in a healthy population would be important to 1) better understand how tDCS may lead to changes in somatosensory processing, 2) provide a rationale for potential therapeutic use of tDCS in the treatment of nervous system disorders such as in pain conditions and stroke and 3) help discover how to best apply tDCS in the treatment of nervous system disorders such as in pain conditions and stroke.

Measuring associations between the different abovementioned measures would be important to firstly potentially provide further evidence of their clinical utility and secondly provide further evidence of how to best utilise the measures in a clinical setting.

2.5 Aims

The aims include:

1) To investigate the effects of five consecutive daily sessions (1 session / treatment day) of anodal sensory cortex tDCS on VDT for vibrations (i.e. vibration frequency either 30 or 200Hz) delivered to the distal pad of both third digits in a healthy population.

2) To investigate the effects of five consecutive daily sessions (1 session / treatment day) of anodal motor cortex tDCS in a healthy human population on:

a)_psychophysical thresholds (i.e. electrical, thermal and mechanical detection and pain thresholds),

b)_subjective pain intensity scores (i.e. electrical, thermal and mechanical pain visual analogue scales (VAS),

c)_objective salivary biomarkers (i.e., substance P and cortisol) levels

2.6 Hypotheses

This thesis's hypotheses include:

1_Five consecutive daily sessions (1 session / treatment day) of anodal sensory cortex tDCS results in significantly lowered vibration detection thresholds (VDT) for vibrations (i.e. 30 or 200Hz) delivered to the distal pad of both third digits compared to the effects of sham sensory cortex tDCS in a healthy human population.

2_Five consecutive daily sessions (1 session / treatment day) of anodal motor cortex tDCS results in significantly

a_lower electrical detection thresholds, higher electrical, thermal and mechanical pain thresholds and lowered subjective pain scores (i.e. electrical, thermal and mechanical pain visual analogue scales) compared to the effects of sham motor cortex tDCS in a healthy human population.

b_lower levels of cortisol and substance P after experimental pain stimulation compared to the effects of sham motor cortex tDCS in a healthy human population.

3_Are there correlations between different psychophysical thresholds (i.e. electrical, thermal and mechanical detection and pain thresholds), subjective pain measures (i.e. electrical, thermal and mechanical pain visual analogue scales (VAS); self-reported general pain sensitivity questionnaire (SRGPSQ)) and objective pain related biomarkers (i.e. salivary substance P and cortisol levels) in a healthy human population.

Chapter 3

Study 1 and Study 2

3.0 Study 1

Modulation of sensory cortex function with transcranial direct current stimulation

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3.1 Abstract

Background: Research has investigated single session tDCS induced effects on body sensory detection thresholds. Systematic reviews indicate heterogenous treatment effects for such trials. Increasing tDCS dose may form more consistent tDCS induced effects on sensory detection thresholds.

Objective/Hypothesis: The study's objective was to investigate the effects of five consecutive daily sessions (1 session / treatment day) of sensory cortex tDCS on vibration detection thresholds (VDT) of the upper limbs in a healthy human population. It was therefore hypothesised that the use of tDCS would effectively modulate VDT for vibrations delivered at two frequencies (30Hz and 200Hz) to both upper limbs over time.

Methods: Randomised controlled trial methodology was used to assess VDT before and after five consecutive daily sessions of either active (anodal) or sham tDCS applied to the dominant somatosensory cortex in 29 healthy volunteers (mean age +/- SD = 22.86 +/- 6.78; 15 males and 14 females) using a method of limits protocol. Possible within-subjects (i.e. factor = time) and between-subjects (i.e. factor = treatment) statistical differences were examined using the mixed model analysis of variance (ANOVA).

Results: A mixed model ANOVA demonstrated no significant differences due to treatment alone.

Conclusion: Increasing tDCS dose not increase the consistency/efficacy of tDCS induced effects on vibro-tactile sensitivity in a healthy human population.

3.2 Introduction

Research is exploring the use of non-invasive brain stimulation techniques to induce neuroplasticity for meaningful purposes. tDCS is one such brain stimulatory technique, which involves delivering low amplitude direct current (1-2mA) to the brain via scalp electrodes (Nitsche et al. 2008).

tDCS has previously been shown to alter the excitability of sensory and motor pathways as well as having lasting effects on behavioural aspects of nervous system function in a healthy human population (Rogalewski et al. 2004, Nitsche, Paulus 2001). A systematic review identified that few studies have investigated the effects of sensory cortex tDCS on sensory detection thresholds in a healthy human population (see Chapter 2). The studies were of good methodological quality but the single session tDCS induced percentage change from baseline values were mostly minimal and inconsistent (Grundmann et al. 2011, Ragert et al. 2008).

There is some research that suggests that repeated daily tDCS may have cumulative effects on neuroplasticity induction (i.e. changes to motor pathway excitability and behavior) in a healthy population (Alonzo et al. 2012, Reis et al. 2009). It therefore may be that repeated sensory cortex tDCS sessions are required to yield larger and more consistent effects on sensory thresholds in a healthy population.

The vibration detection threshold (VDT) measure is an objective method of testing human sensory function in research procedures (Stuart et al. 2003). The aims of this research study were two fold:

1_to establish the effects of five consecutive daily sessions (1 session / treatment day) of sensory cortex tDCS on VDT in a healthy human population compared to sham tDCS.

2_to establish whether time influences tDCS induced effects on VDT.

Previous research has demonstrated that anodal tDCS can increase the level of excitability of both the human motor and sensory pathways (Matsunaga et al. 2004, Nitsche, Paulus 2000, Sugawara et al. 2015). It was therefore hypothesised that consecutive daily sessions of anodal sensory cortex tDCS would effectively lower VDT compared to sham over time. Investigating tDCS induced effects on VDT in a healthy human population would provide further evidence for the effectiveness of tDCS as a tool to manipulate cortical plasticity, which could translate into advanced treatment for populations characterised by sensory cortex function abnormalities.

3.3 Material and methods

3.3.1 Study design

A prospective randomised single blinded controlled trial was instituted involving one experimental and one sham control group.

With respect to randomisation, subjects were allocated to their respective groups through random concealed allocation. The randomisation procedure involved concealed drawing pieces of paper, which had a noted intervention (i.e. active or sham).

With respect to blinding, participants were not told what intervention group they belonged to. The same investigator administered the brain stimulation and recorded the VDT. The investigator could not be blinded due to limitations in resources to finance equipment or additional personnel to enable blinding of the investigator.

3.3.2 Setting

The following research was carried out in a quiet, controlled and appropriate University research laboratory.

3.3.3 Sample size power calculation

An a priori sample size power analysis was used to calculate required sample size to test ANOVA within-subjects factor (6x time points) and between-subjects factor (2x treatment group) interactions. Using G*Power software, eta-squared can be used to calculate effect size (f) for ANOVA (Prajapati, Dunne & Armstrong 2010). Aslaksen, Vasylenko and Fagerlund (2014) previously reported eta-squared values in the range of .07 to .33 for significant tDCS induced effects on experimental pain in a healthy human population. Considering 95% statistical power, a two sided α =.05 and a 'moderate' effect size = 0.27 a total of n=24 were required (Faul et al. 2007, Cohen 1992).

3.3.4 Participants

Twenty nine subjects were allocated to an experiment group and a control group consisting of 14 and 15 subjects respectively. Table 8 reveals participant flow information. The subjects in the experimental group received anodal sensory cortex tDCS. Subjects were recruited from the staff and

student population at Bond University, Australia. Table 9 reveals participant demographic information. Participants were given written and oral information regarding the investigation (see Appendix 1). Persons were excluded from participation if they: had any metallic or magnetic pieces inside the brain/skull (except titanium); had any implanted metal devices; had epilepsy or have ever experienced a convulsion or seizure; had any first degree relatives with epilepsy; had any hearing problems or tinnitus; consume heavy amounts of alcohol (e.g. +4 standard drinks/day) very regularly; had any recent or severe heart disease or were possibly pregnant (see Appendix 2). The participants were recruited between July 2012 and May 2013. Participation was voluntary and all subjects provided written informed consent prior to inclusion into the study (see Appendix 3). The study was approved by the Bond University Human Research Ethics Committee (RO1439) and carried out in accordance with the Declaration of Helsinki.

Table 8 Participant flow information

Participant flow variable	**Value**	**Reasoning**
Advertisement responders	n = 90	1_Time commitment 2_Ineligible
Non-completed participants	n = 8	1_Not able to do VDT 2_Dropped out
Completed participants	n = 29	
Completed participants not analysed	n = 1	Data appeared to be outlier

Table 9 Participant demographic information

Variable	Value
Sex	
Male	15
Female	14
Handedness	
Right	24
Left	5
Age (mean in yrs +/- SD)	22.86 (+/- 6.78)
Range	18-45

3.3.5 Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) was applied using a low intensity direct current stimulator (Chattanooga Ionto, Tennessee, USA) and delivered via scalp electrodes prepared as follows: Household sponges (thickness = 10mm, contact area = 35cm^2) were soaked in electrolyte solution (NaCl =154mM) and attached to each side of an aluminium foil sheet (area = 35 cm^2) with a rubber band. The anode was positioned over the sensory cortex at either the C3' or C4' position, which correlated to 2 cm posterior to the C3 or C4 position (10-20 EEG system) of the subject's dominant cortex (Ragert et al. 2008). The cathode was placed over the contralateral supra-orbital region (Ragert et al. 2008). The electrodes were maintained in position by a non-conducting elastic strap, which was strapped firmly around the subject's head (Norris, Degabriele & Lagopoulos 2010). For each session, tDCS was delivered at a current intensity of 1mA (current density of .02857 mA/cm^2) for 20 minutes. The current density, polarity, and duration of tDCS

that was applied in this study have all previously been shown to influence somatosensory processing in a healthy population (Boggio et al. 2008).

To quantify any placebo effect there was a control group, which received sham stimulation only. This involved activating the tDCS device at a current intensity of 1mA but turning the tDCS device off slowly, out of the subject's field of view, after ~30 seconds (Gandiga, Hummel & Cohen 2006). The sham procedure chosen was based on research that demonstrated that ≤ two minutes of tDCS at a current intensity of .02857 mA/cm2 delivered to the motor cortex was insufficient to induce alterations post-stimulation to motor pathway excitability (Nitsche, Paulus 2000). This approach has previously been proven to be reliable at 1 mA for both naive and experienced subjects (Ambrus et al. 2012). Stimulation followed the current published guidelines for safe use (Nitsche et al. 2008).

3.3.6 Outcome measure

3.3.6.1 Vibration Psychophysical thresholds

This study specifically looked at the ability to detect sinusoidal vibrations, which were vertical uni-planar, periodical oscillations applied to the skin surface. A signal generator software program (AD Instruments, LabChart 7, Australia) generated the sinusoidal waveforms, which were then passed to a linear power amplifier (Gearing and Watson, PA30, UK) before being delivered to the skin surface via a perspex probe (6-mm-diameter) attached to the shaft of a mechanical vibrator (Gearing and Watson, GWV4, UK). The mechanical vibrator was mounted on an isolated rigid trunnion (Gearing and Watson, T4, UK). The software controlled alterations to both the frequency

and voltage amplitude of the sinusoid waveforms. This type of vibration system has been used in similar research to the present study (Morley et al. 2007).

As the mechanical vibrator system is not feedback controlled, offline calibrations were made using a hydraulic micromanipulator (Narishige, MHW-103, Japan) to identify the amplitude of vibration that is produced (in microns) with known settings on the signal generator/amplifier system.

The subjects were seated upright in a chair and in parallel to the length of a rectangular table, which stationed the mechanical vibrator. Foam blocks on the table stabilised the subject's upper limb and helped to keep the hand in a pronated position. A measuring tape was used to ensure the same distance between foam block and mechanical vibrator for each VDT assessment. The investigator then lined the centre of the subject's distal pad of the third digit on the vibrator's probe tip, which was flush with a 6mm hole in a rigid perspex plate (surface area = 30 cm^2) suspended from the rigid trunnion. The plate limits the spread of surface waves across the skin, and helped to maintain a constant indentation of the probe in the skin of the testing site (Stuart et al. 2003). The probe and the rigid surround were separated by a gap of 2mm. A measuring tape was used to ensure that the same site of stimulation was used between sessions. The subject was instructed to keep their finger in soft contact with the stimulating probe for the testing. Subjects also wore earmuffs to avoid any potential auditory cues from the vibration device.

Vibrations were delivered specifically to the distal pad of the third digit of both hands at two different frequencies (30 & 200Hz). These frequencies were

chosen to selectively activate different sensory receptors (Kandel, Schwartz & Jessell 2000). 30Hz vibrations preferentially activated Meissner corpuscles, whereas 200Hz vibrations activated mainly Pacinian corpuscles (Kandel, Schwartz & Jessell 2000). Both upper limbs were assessed to measure both the contralateral (dominant side) and ipsilateral (non-dominant) side responses to brain stimulation of the dominant hand representation (refer to Table 10). Site has previously been shown to have influence on tDCS induced effects on VDT (Jürgens et al. 2012).

Table 10 VDT measures

Parameter	**Outcome measure**
	Psychophysical
Vibration	Dominant_200Hz (D200)
	Non-dominant_200Hz (ND200)
	Dominant_30Hz (D30)
	Non-dominant_30Hz (ND30)

VDT was assessed using a method of limits technique (Stuart et al. 2003). For each frequency, subjects initially experienced a randomly chosen supra-threshold vibratory stimulus. The stimulus amplitude was then gradually decreased (descending mode) at a constant rate (~1s / stimulus amplitude) until the subject verbally indicated that they could confidently no longer detect it. After this, the vibratory stimulus was then gradually increased at a constant rate (~1s / stimulus amplitude) from a randomly chosen sub threshold level (ascending mode) until the subject verbally indicated that they could confidently detect the vibration stimulus. The mean of a minimum of 10 detection thresholds (five ascending and descending) for each frequency and

upper limb was calculated for each subject (Stuart et al. 2003). The method of limits procedure was selected for measuring vibro-tactile sensitivity as it has previously been shown to be more reliable and time efficient than the forced choice procedure (Gerr, Letz 1988).

With respect to timing, VDT was objectively measured both before and after tDCS during the first, third and final sessions (i.e. total VDT time points = 6). Baseline (i.e. pre-tDCS) VDTs were measured only at time point 1. The outcome measures of VDT at each time point included: dominant-30Hz, dominant-200Hz, non-dominant-30Hz and non-dominant-200Hz. A practice session was also incorporated on day 1. All the measurements were performed between 7:45am and 5:30pm. The experimental procedure is shown diagrammatically in Figure 2.

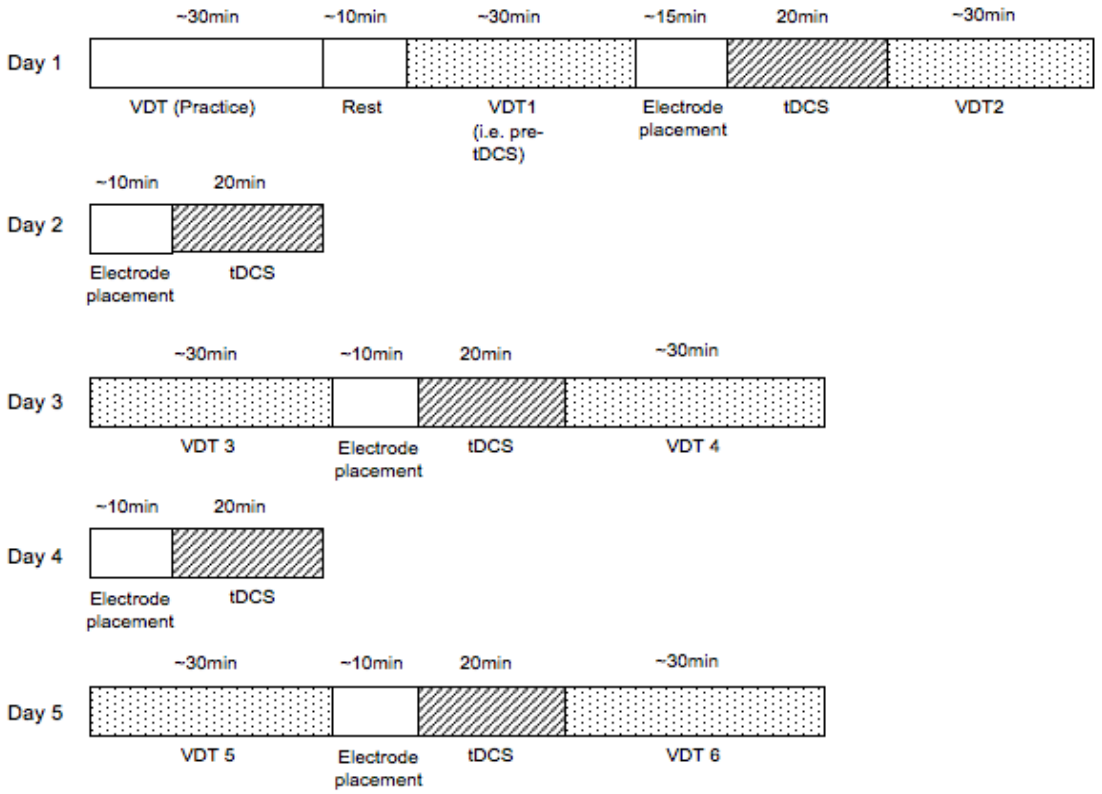

Figure 2 Study design, showing the time course of tDCS treatments and VDT measurements. tDCS treatments (20 mins) were delivered once per day for 5 consecutive days. Vibration detection thresholds (VDT) (for 30Hz and 200Hz and for both dominant and non dominant arms) were measured before and after tDCS on days 1, 3 and 5.

3.3.7 Data analysis

Pooled non-transformed VDT means were produced in order to compare means with previous literature.

The primary analysis endpoint (i.e. primary outcome measure) for this study was the dominant 200Hz variable outcome measure. The non-dominant 200Hz, dominant 30Hz and non-dominant 30Hz variable outcome measures were secondary analysis endpoints.

A mixed model analysis of variance (ANOVA) statistical test analysis was chosen to test repeated vibration detection threshold measures at six time points (see Figure 2) in response to either one of two interventions (active or sham tDCS). The time factor represents the “within-subjects” factor, while the treatment group is the “between-subjects” factor. Our research hypothesis was that there will be a significant interaction effect, and that the subjects in the active tDCS would have a greater change over time in the between-subjects factor.

In the presence of a significant interaction, the analysis was refined by using the syntax features of SPSS to allow a simple main effects analysis with Sidak post hoc test for the interaction effect (Peat, Barton 2014).

Sidak adjustment was used for repeated measures of individual test condition (e.g. dominant 200Hz), as Sidak adjustment is not affected as much by loss of statistical power for which Bonferroni adjustments are affected by (Dmitrienko, D’Agostino 2013).

If the interaction effect between the within-subjects and between-subjects factor was not significant, the interpretation of the analysis was reverted to interpreting the main effects for both factors (i.e., the "within-subjects" factor and "between-subjects" factor). In addition, if the main effect of time was statistically significant, output from Sidak post hoc tests were interpreted to understand where the differences between factors lie.

Mixed models analysis of variance requires the following assumptions to be satisfied:

• The assumption of normality for the repeated measures

• The assumption of sphericity for the within-subjects factor for the repeated measures

• The assumption of homogeneity of variance for the between-subjects factor

The standardised residuals were therefore checked to determine if they were approximately normally distributed, through Shapiro-Wilk's test for normality or visually through histograms.

The homogeneity of variance assumption was assumed if Fmax was less than 10 or Levene's test of equality of error variances was not significant (sig if $p < .05$) (Tabachnick, Fidell 2007).

Huyn-Feldt or Greenhouse-Geisser Epsilon corrections were used if Mauchly's test for sphericity was significant. Greenhouse-Geisser Epsilon correction was used if the estimated epsilon was <0.75 whereas Huyn-Feldt Epsilon correction was used if the estimated epsilon was >0.75.

Partial eta-squared (indicated by η_p^2) was used as an estimated measure of effect size (Allen, Bennett 2012).

An independent samples t-test was used to compare mean VDT between groups at baseline to ensure equivalent baseline characteristic between groups after randomisation had occurred.

Percentage change from baseline following 1 and 5 tDCS sessions within groups was also assessed. A percentage change from baseline value assessment was performed to enable comparisons in findings with recent systematic reviews (Vaseghi, Zoghi & Jaberzadeh 2014).

A p-value of ≤0.05 was considered significant for significance tests. For each analysis, IBM SPSS 20.0 for Windows was used.

3.4 Results

Pooled non-transformed VDT means (± standard error of the mean) were produced (refer to Figure 3).

D200, ND200 and D30 violated the assumption of normality and were therefore transformed (i.e. reciprocally or logarithmically) in order to meet the normality assumption (refer to Table 11).

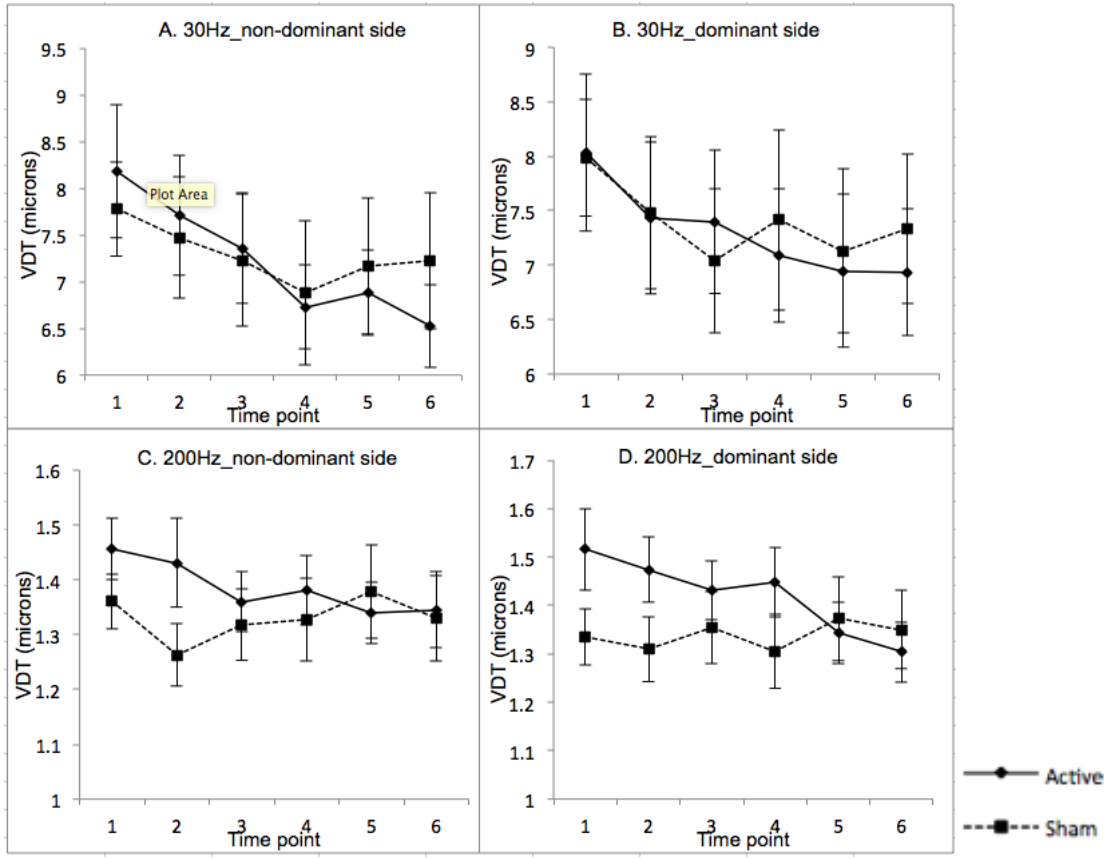

Figure 3 Pooled mean VDTs at each time point for vibrations delivered at a) 30Hz to the non-dominant upper limb, b) 30Hz to the dominant upper limb, c) 200Hz to the non-dominant upper limb and d) 200Hz to the dominant upper limb.

Table 11 The effects of sensory cortex tDCS on vibration detection thresholds

Parameter	Measure	Test	F	Sig.	η_p^2
	Psycho-Physical				
Vibration	D200[a]	Group	1.4	.25	.048
		Time	2.9	**.04**	.100
		Time x Group	4.2	**.01**	.136
	ND200[b]	Group	.73	.40	.026
		Time	1.7	.16	.059
		Time x Group	2.0	.11	.068
	D30[b]	Group	.001	.98	< .001
		Time	2.2	.07	.077
		Time x Group	.25	.90	.009
	ND30	Group	.01	.94	< .001
		Time	2.5	**.03**	.085
		Time x Group	.49	.79	.018

[a] = reciprocally transformed
[b] = logarithmically transformed
Bold text p<0.05

Table 12 VDT significant post hoc comparisons

Parameters	Measure	Pairwise Comparison	Mean difference (non-transformed)	Sig.	95% CI
	Psycho-Physical				
Vibration	D200	1 vs. 6	.212	.03	-.195 to -.006
		2 vs. 6	.170	.03	-.169 to -.006
		3 vs. 6	.128	.01	-.126 to -.014

The ANOVA demonstrated no statistically significant between (i.e. factor = treatment) group differences (refer to Table 11). In contrast, the ANOVA demonstrated statistically significant within-subjects (i.e. factor = time) differences for D200 ($p = .04$) and ND30 ($p = .03$). The partial eta-squared effect size was medium for the effect of time for D200 and ND30. In line with marginal significance, post hoc pairwise comparisons were not significant following Sidak adjustment. However, the ANOVA also showed a statistically significant time x group interaction for D200 ($p = .01$). Certain post hoc comparisons were significant and demonstrated that there was a significant lowering in D200 at time point 6 compared to time points 1 ($p = .03$), 2 ($p = .03$) and 3 ($p = .01$) for active tDCS only (refer to Table 12). The partial eta-squared effect size was medium for the interaction effect for D200.

No statistically significant differences in baseline mean VDT between the groups (active – sham) was observed.

The range of the pooled mean percentage change from baseline values at time points 2 and 6 is listed below:

- Time point 2:
    - Anodal; -2.1 to -7.5
    - Sham; -2.2 to -7.4

- Time point 6:
    - Anodal; -8.2 to -20.7
    - Sham; -8.1 to +0.7

3.5 Discussion

With respect to detection thresholds, the mean detection thresholds obtained in this study for both high and low frequency vibrations were smaller than those obtained by Stuart et al. (2003) but comparable to results reported by Morley and Rowe (1990). Direct comparisons of thresholds described here and those reported by others using a similar vibration set up are difficult due to methodological differences such as contact conditions. VDTs for vibrations delivered at both high and low frequencies to the finger can be affected by contact conditions such as the stimulation probe size and the size of the gap between contactor and rigid surround (Morioka, Whitehouse & Griffin 2008). If we compare the present study vibration set up to Stuart et al. (2003), the stimulation probe size was bigger and the size of the gap between contactor and rigid surround was smaller.

With respect to the first aim of this study that compared the effects of consecutive daily sessions of tDCS on VDT compared to sham tDCS, the results demonstrated no statistical between-group differences. This is in line with the literature where previous studies have failed to show an effect of a single session tDCS on VDT compared to sham tDCS in a healthy population (Bachmann et al. 2010, Grundmann et al. 2011). More stringent methodology for measuring vibro-tactile sensitivity such as using a software controlled mechanical vibrator instead of tuning forks and using a method of limits approach for sensory testing instead of only vibration disappearance thresholds could increase the validity of results. The findings are also in line with recent literature that failed to show an effect of a single session tDCS on

vibration discrimination thresholds compared to sham tDCS in a healthy population (Hanley, Tommerdahl & McGonigle 2015).

The findings therefore ultimately suggest that increasing stimulation frequency (i.e. using repeated daily tDCS) appears not to influence the effectiveness of tDCS on VDT in a healthy population. This is therefore not in line with previous literature that suggested that repeated daily tDCS might have cumulative effects on neuroplasticity induction in a healthy population (Alonzo et al. 2012, Reis et al. 2009).

Reasons why there was a lack of significant tDCS induced effects on vibro-tactile sensitivity reported in our study may be due to key methodological and study design limitations. These include intervention related limitations, intervention timing, population type and outcome measure settings and timing. Discussion around these parameters is further detailed in Chapter 4.

Exactly how tDCS may affect sensory function in a healthy population is not fully understood. tDCS has previously been shown to alter the excitability of the sensory cortex in a polarity-dependent manner (Dieckhöfer et al. 2006). tDCS induced changes to sensory behavior may therefore be the result of alterations to task specific cortical networks, which may involve changes to synaptic efficiency and the level of cortical excitability within the stimulated body part representation in the somatosensory cortex (Nitsche et al. 2004, Tegenthoff et al. 2005). Further research appears to be required before firm conclusions can be made regarding the mechanisms of tDCS post stimulus effects on somato-sensory processing.

With respect to the secondary aim of this study, the results demonstrated that there was a significant effect of time for tDCS induced effects on ND30 and D200. However, in the case of D200, the effect of time was only significant depending on group. These findings agree and disagree with previous literature that did or did not show an effect of time for single session tDCS on VDT (Bachmann et al. 2010, Grundmann et al. 2011). However, it can be seen that for both active and sham tDCS groups there was a steady reduction in 30Hz VDTs for both sides (i.e. ipsilateral and contralateral to tDCS) over time (refer to Figure 3). These findings possibly suggest that a training effect may have been present for 30Hz VDT. Hence, further research is required that more appropriately takes into account potential training effects before stronger conclusions surrounding the influence of time on tDCS induced effects on VDT can be made.

Both upper limbs were assessed to measure both the contralateral (dominant side) and ipsilateral (non-dominant) side responses to brain stimulation of the dominant hand representation. It is important to note that outcome measure side (i.e. ipsilateral vs. contralateral to tDCS) has been shown to have influence on tDCS induced effects on VDT (Jürgens et al. 2012). In addition, it is known that 30 & 200Hz vibratory stimuli selectively activate different sensory receptors (Kandel, Schwartz & Jessell 2000). Vibrations of 30Hz preferentially activate Meissner corpuscles, whereas 200Hz vibrations have been reported to mainly activate Pacinian corpuscles (Kandel, Schwartz & Jessell 2000). Although the research did not primarily aim to establish which side (i.e. dominant or non-dominant) or frequency of vibration have influence on tDCS induced effects on VDT, the results suggest that side and frequency

of vibration may have influenced tDCS induced effects on VDT. For example, a statistically significant effect of time was found on one side only for each vibration frequency. Further research is required before conclusions relating to the influence of outcome measure side or receptor activation on tDCS induced effects on VDT can be drawn.

With respect to percentage change from baseline, values following 1 or 5 tDCS sessions in this study were generally minimal in the negative direction. However, percentage change from baseline values following anodal tDCS were slightly higher in magnitude than those following sham tDCS. These findings are similar to previously reported single session sensory cortex tDCS induced percentage change from baseline mechanical sensory threshold changes (see Chapter 2). Interestingly, percentage change from baseline values following anodal tDCS were slightly higher in magnitude at time point 6 compared to time point 2.

3.6 Limitations

There are a number of methodological issues that need to be addressed. Firstly, the study was conducted on predominantly a young university student population. Hence, the results from this study may not necessarily translate to other age groups. Secondly, the outcome measures were also performed only at one anatomical location (i.e. finger). The results from this study may therefore also not necessarily translate to other body parts (e.g. lower limb). Thirdly, due to limitations in resources to finance equipment or additional personnel, the participants were blinded to treatment group but the researcher

was not blinded. Obviously, this increased possibility for bias. Finally, Sidak adjustment was used for repeated measures of separate test conditions, as it is not affected as much by loss of statistical power for which Bonferroni adjustments are affected by. It can be argued that the 4 test conditions can be considered as separate entities and therefore not requiring further restrictive multiplicity penalisation of the model (Dmitrienko, D'Agostino 2013).

3.7 Conclusion

In summary, this is the first study that has demonstrated that consecutive daily sessions of sensory cortex tDCS cannot consistently modify vibro-tactile sensitivity in a healthy human population compared to sham tDCS. The findings are in line with previous single session tDCS literature therefore ultimately suggesting that increasing tDCS dose (i.e. repeated daily) does not overtly influence end results.

3.8 Study 2

Modulation of experimental pain perception by transcranial direct current stimulation in healthy adults.

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3.9 Abstract

Background: A number of trials have explored the effects of single session tDCS induced effects on pain related measures in a healthy population. Recent systematic reviews point out heterogenous treatment effects for such trials. Multiple tDCS dose strategies may help deliver more efficacious tDCS induced effects on such measures.

Aims: The aims of the study were twofold. The primary aim was to investigate the potential effects of five consecutive daily sessions (1 session / treatment day) of motor cortex tDCS on both psychophysical thresholds (e.g. sensory detection and pain thresholds in response to electrical, pressure and thermal stimulation of the body) and subjective pain intensity scores (i.e. electrical, pressure and thermal body pain visual analogue scales (VAS)) in a healthy human population. The secondary aims of the study included firstly to investigate the effects of five consecutive daily sessions of tDCS on objective pain related hormones/neuropeptides (i.e. salivary cortisol, substance P) after experimental pain stimulation and then to explore the correlations between baseline psychophysical, subjective and objective measures.

Methodology: Randomised controlled trial methodology was used to assess five consecutive daily sessions of either active (anodal) or sham dominant motor cortex tDCS on pain related psychophysical, subjective and objective outcome measures in 42 healthy volunteers (mean age +/- SD = 24.95 +/- 7.22; males = 14, females = 28). Possible within-subjects (i.e. factor = time) and between-subjects (i.e. factor = treatment) statistical differences for psychophysical and subjective outcome measures were examined using the

mixed model ANOVA. Possible within- and between-group differences for the objective outcome measure were analysed using statistical t-tests. Cross-sectional analysis of baseline data was also used to explore bivariate correlations between examined baseline outcome measures.

Results: A statistically significant between-subjects difference was observed when comparing the mean psychophysical threshold at all time points for one psychophysical threshold variable (i.e. CPT) only. The group mean estimates indicated that the active group had higher transformed CPT compared to the sham group. ANOVA demonstrated no statistically significant within-subjects differences as well as no significant time x treatment interaction effect. Results showed no statistically significant between-group differences for the objective outcome measure. Statistically significant correlations between psychophysical and subjective baseline outcome measures were found.

Conclusions: Increasing stimulation dose (e.g. repeated daily) does not consistently influence anodal motor cortex tDCS effects on experimental pain perception. As well, the results of the study may provide further evidence of the clinical utility of different types of pain assessments.

3.10 Introduction

Advancements in neuroscience methods have led to increasing use of non-invasive brain stimulation techniques in neuroscience research. One of these techniques, namely transcranial direct current stimulation (tDCS), can achieve nervous system modulation by delivering a weak (<2mA) direct current via surface electrodes (see (Nitsche et al. 2008) for review).

Psychophysical measures can give an indication of sensory and pain threshold changes while self-report scales (i.e. numeric or visual analogue) can allow subjective rating of pain intensity (Bachmann et al. 2010, Boggio et al. 2008, Jürgens et al. 2012). Measuring tDCS induced effects on these measures, in a healthy population, provides a rationale for potential therapeutic use of tDCS in the treatment of pain.

A systematic review identified that a number of studies have investigated the effects of motor cortex tDCS on pain related psychophysical and subjective measures in a healthy human population (see Chapter 2). The studies were of good methodological quality but the single session tDCS induced percentage change from baseline values were mostly minimal and inconsistent for psychophysical measures and mostly moderate (i.e. 20-30%) for subjective measures.

Research has previously indicated cumulative effects on neuroplasticity induction (i.e. relating to changes to motor pathway excitability and behavior) following the use of repeated daily tDCS in a healthy population (Alonzo et al. 2012, Reis et al. 2009). Larger and more consistent effects on pain related psychophysical and subjective measures in a healthy population may therefore require the use of repeated daily motor cortex tDCS.

Consequently, the aims of this study were twofold:

1_The primary aim was to investigate the potential effects of five consecutive daily sessions (1 session / treatment day) of tDCS on psychophysical (i.e. electrical, thermal and mechanical detection and pain thresholds) and subjective (i.e. electrical, thermal and mechanical

pain visual analogue scales) measures in a healthy human population. Nitsche and Paulus (2001) demonstrated that anodal tDCS can increase the level of human motor pathway excitability. It was therefore hypothesised that consecutive daily sessions of anodal tDCS would effectively result in a greater lowering of sensory detection thresholds and subjective pain ratings, as well as a greater increase of pain thresholds over time compared to sham.

2_The secondary aims of the study included firstly to investigate the effects of five consecutive daily sessions of tDCS on objective pain related hormones/neuropeptides (i.e. salivary cortisol, substance P) after experimental pain stimulation and then to explore the correlations between baseline psychophysical, subjective and objective measures. Anodal tDCS has previously been shown to lower cortisol compared to sham tDCS (Binkofski et al. 2011). It was therefore also hypothesised that anodal tDCS would lower levels of cortisol and substance P after experimental pain stimulation compared to the effects of sham motor cortex tDCS in a healthy human population

3.11 Material and methods

3.11.1 Study design

A prospective randomised single blinded controlled trial was instituted involving one experimental and one sham control group.

With respect to randomisation, subjects were allocated to their respective groups through random concealed allocation. The randomisation procedure

involved concealed pieces of paper, which had a noted intervention (i.e. active or sham). However, stratification of gender between groups was not implemented before randomisation.

3.11.2 Setting

The following research was carried out in a quiet, controlled and appropriate University research laboratory.

3.11.3 Sample size power calculation

An a priori sample size power analysis was used to calculate required sample size to test ANOVA within-subjects factor (3xtime points) and between-subjects factor (2xtreatment group) interactions. Using G*Power software, eta-squared was used to calculate effect size (f) for ANOVA (Prajapati et al. 2010). Aslaksen, Vasylenko and Fagerlund (2014) previously reported eta-squared values in the range of .07 to .33 for significant tDCS induced effects on experimental pain. Considering 95% statistical power, a two sided $\alpha = .05$ and a 'moderate' effect size = 0.27 a total of n=36 were required (Faul et al. 2007, Cohen 1992).

3.11.4 Participants

Table 13 reveals participant flow information. Subjects (n=42) were allocated into an experiment (n=24) group and a control (n=18) group. The subjects in the experimental group received anodal tDCS. Subjects were recruited from the staff and student population at Bond University and the Gold Coast community. Table 14 reveals participant demographic information. Participants were given written and oral information regarding the investigation (see Appendix 4). People were excluded from participation if

they: had any metallic or magnetic pieces inside the brain/skull (except titanium); had any implanted metal devices; had epilepsy or have ever experienced a convulsion or seizure; consume heavy amounts of alcohol (e.g. +4 standard drinks/day) very regularly; had any recent or severe heart disease or were possibly pregnant (see Appendix 5). Participation was voluntary and all subjects provided written informed consent prior to inclusion into the study (see Appendix 6). The participants were recruited between September 2013 and June 2014. The study was approved by the Bond University Human Research Ethics Committee (RO1693) and carried out in accordance with the Declaration of Helsinki.

Table 13 Participant flow information

Participant flow variable	**Value**	**Reasoning**
Advertisement responders	n = 142	1_Time commitment 2_Ineligible
Non-completed participants	n = 1	Participant dropped out
Completed participants	n = 41	
Completed participants not analysed	n = 1	pH indicator revealed a saliva sample that was acidic

Table 14 Participant demographic data

Variable	Value
Sex	
Male	14
Female	28
Handedness	
Right	38
Left	4
Age (mean in yrs +/- SD)	24.95 (+/- 7.22)
Range	18-46

3.11.5 Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) was applied using a low intensity direct current stimulator (Chattanooga Ionto, Tennessee, USA) and delivered via scalp electrodes prepared as follows: Household sponges (thickness = 10mm, contact area = 35cm2) were soaked in electrolyte solution (NaCl =154mM) and attached to each side of a carbon rubber electrode (area = 35 cm^2) with a rubber band. The anode was positioned at either the C3 or C4 position (10-20 EEG system) of the subject's dominant cortex. The cathode was placed over the contralateral supra-orbital region (Ragert et al. 2008). The electrodes were maintained in position by a non-conducting head strap, which was strapped firmly around the subject's head (Norris, Degabriele & Lagopoulos 2010). For each session, tDCS was delivered at a current intensity of 2mA (current density of .0571 mA/cm^2) for 30 minutes. The current density, polarity, and duration of tDCS that was applied in this study

have all previously been shown to influence somatosensory processing in a human population (Fregni et al. 2006b).

To quantify any placebo effect there was a control group, which received sham stimulation only. This involved activating the tDCS device at a current intensity of 2mA but turning the tDCS device off slowly, out of the subject's field of view, after ~30 seconds (Gandiga, Hummel & Cohen 2006). The sham procedure chosen was based on research that demonstrated that < two minutes of tDCS at a current intensity of .02857 mA/cm^2 delivered to the motor cortex was insufficient to induce alterations post-stimulation to motor pathway excitability (Nitsche, Paulus 2000). Stimulation followed the current published guidelines for safe use (Nitsche et al. 2008).

3.11.6 Outcome Measures

The outcome measures used for this study are arranged into 3 study objectives in line with the primary and secondary aims mentioned previously. Thus the following sections are as follows:

1) tDCS effects on psychophysical threshold and subjective VAS measures (refer to Table 15)

2) tDCS effects on objective measures (refer to Table 15) and

3) correlations between baseline psychophysical threshold, subjective and objective measures.

The proceeding data analysis, result and discussion sections are similarly categorised.

Table 15 Psychophysical, subjective and objective outcome measures

Parameters	**Outcome measures**		
	Psychophysical	Subjective	Objective
Electric	EDT		Cortisol
	EPT	EPT_VAS	Substance P
Mechanical	PPT	PPT_VAS	
Thermal	CPT	CPT_VAS	
	CTT	CTT_VAS	

3.11.6.1 tDCS effects on psychophysical threshold and subjective VAS measures

This study specifically looked at sensory detection and pain thresholds to electrical, mechanical pressure and thermal stimuli. The International Association for the study of Pain definitions of pain and pain thresholds were used as a framework for our pain threshold measurements. Thresholds to electrical, mechanical pressure and thermal stimuli were assessed using a method of limits technique in order to minimize number of stimuli per pain measurement (Boggio et al. 2008, Rolke et al. 2006, Neziri et al. 2011b).

3.11.6.1.1 Electric psychophysical thresholds

A computer software program (AD Instruments, Lab Chart 7, Australia) was used to generate constant current electrical pulses (pulse duration = 200 microseconds; maximum repetition rate = 100Hz). The electrical pulses were then passed to a built-in isolated stimulator (PowerLab, Ad Instruments, Model No. ML-856) for isolation from the mains power and delivered to the site of stimulation (i.e. skin surface of the dominant index finger) via a

stimulating bar electrode (i.e. 2x 9 mm diameter circular skin contacts, which were spaced 30 mm apart). The pulse duration that was applied in this study has previously been used to demonstrate tDCS induced effects on sensitivity to electrical body pain stimuli in a healthy population (Boggio et al. 2008). The pulse frequency that was applied in this study has previously been used to assess thresholds for electrical pain stimuli (Laitinen, Eriksson 1985).

Subjects were seated in front of a table that stationed the electrical stimulator set up. To ensure a low impedance electrode contact, the tester then 1) applied a small amount of electrode paste (Ten20, D.O. Weaver and CO., USA) to each stimulating bar electrode skin contact and 2) cleaned the subject's dominant index finger's skin surface using an alcohol swab. The tester then placed the subject's dominant index finger on the stimulating bar electrode skin contacts. The subject was then instructed to keep their finger in contact with the stimulating bar electrode using a small amount of force. The subject was then blindfolded and wore earmuffs to avoid any potential auditory or visual cues from the stimulator set up.

Prior to application of the electrical stimuli, the subject was told that they would need to verbally state 1) when they could first confidently 'detect' the electrical stimulus and 2) when they could first confidently perceive the electrical stimulus as being 'a prickly sharp unpleasant painful' sensation. The tester then controlled alterations to the amplitude (i.e. mA) of the electrical pulses using the abovementioned computer software. Current supply started at 0 mA and was increased in steps of 0.1 mA (application rate = .1 mA / 1 seconds) until the subject could confidently first perceive the stimulus as being 'painful'.

The current amplitudes (i.e. mA) when the subject verbally stated that they could 1) first confidently 'detect' the electrical stimulus (i.e. electrical detection threshold = EDT) and 2) first confidently perceive the electrical stimulus as being 'prickly sharp and painful' (i.e. electrical pain threshold = EPT) were then recorded. The trial was repeated for a total of three times. Each trial, however, was separated by a ~30 second non-stimuli period in order to prevent 'wind-up' (Eide 2000). The mean of 3 'detection' and 'pain' thresholds could then be calculated.

3.11.6.1.2 Mechanical psychophysical thresholds

A hand held computerised pressure algometer (AlgoMed, Israel) was used to generate the pressure stimuli that were delivered to the skin surface of the subject's dominant hand (i.e. base of thumb) via a stimulation probe (shape = circular; surface area = $1cm^2$).

The subjects were seated in parallel to the length of a table, which stationed the pressure algometer setup. The subjects were instructed to rest their dominant upper forelimb on the table, which assisted in stabilising the subject's upper limb and helped to keep the hand in a supine position. The subject was then blindfolded and wore earmuffs to avoid any potential auditory or visual cues from the stimulator set up.

Prior to application of the mechanical pressure stimuli, the subject was told that they would need to physically activate a hand held response unit (AlgoMed, Israel) with their non-dominant hand when they could first confidently perceive the pressure stimulus as being an 'unpleasant painful pressure' sensation. To avoid lateral rotation of the subject's dominant upper forelimb during pressure application, the tester held the subject's thumb down

to the table using his non-dominant hand. The tester then placed the pressure algometer stimulating probe on the stimulation site (i.e. base of thumb, thenar eminence). The tester then manually controlled alterations to the amplitude (application rate = ~10 kPa/ second) until the subject physically activated the hand held response unit. The pressure application rate that was applied in this study was chosen to prevent low false threshold results (Jensen et al. 1986). The tester looked at the computerised pressure algometer software program for real-time visual feedback of the pressure application rate in order to maintain pressure application rate throughout pressure application.

The mechanical pressure amplitude (i.e. kPa) at the point when the subject physically activated the hand held response unit (i.e. pressure pain threshold = PPT) was then recorded. The trial was repeated for a total of three times. Each trial, however, was separated by a ~30 second non-stimuli period. The mean of 3 'pain' thresholds could then be calculated.

3.11.6.1.3 Thermal psychophysical thresholds

A cold pressor test was used to measure sensitivity to thermal body pain stimuli delivered to the subject's dominant hand. The tester instructed the subject to stand next to a table that stationed a plastic container filled with ice saturated water (temperature of water = 0-1 degrees celsius) and then marked the subject's wrist on their dominant upper limb using a marker pen. The water temperature that was used in this study has previously been used to assess thresholds for the cold pressor test (Neziri et al. 2011b).

Prior to the application of thermal stimuli, the subject was told that they would be required to verbally state when they could first confidently perceive the stimulus as being 'a cold unpleasant painful' sensation. The subject was also instructed to withdraw their hand when they could no longer tolerate the

stimuli. The subject was further instructed to not 1) clench their fist during the cold pressor test, 2) move their hand during the cold pressor test, 3) submerge their arm in the ice saturate water (i.e. hand only) and 4) perform the test for more than two minutes for consistent administration of the task and measurement (Von Baeyer et al. 2005). The subject then placed their hand into the ice-saturated water to the level of their wrist markings.

The time duration (i.e. seconds) when the subject 1) first stated that they could confidently perceive the stimulus as being a 'cold unpleasant painful' sensation (i.e. cold pain threshold = CPT) and 2) withdrew their hand from the ice saturated water (i.e. cold tolerance threshold = CTT) was recorded. A single 'pain detection' threshold and 'pain tolerance' threshold could therefore be established.

3.11.6.1.4 Subjective pain visual analogue scales

To analyse self-reported experimental body pain intensity, a visual analogue scale (VAS) with a single line and scoring range from 0 for 'no pain' to 100mm for 'pain as bad as you can imagine' was used. After each individual experimental body pain trial, the subject placed a vertical mark on the line to indicate the intensity of pain when they first perceived the stimulus as being painful or could no longer tolerate the stimuli (see Appendix 7). A pain visual analogue scale score could therefore also be calculated for each psychophysical threshold measurement. The VAS is the expert advisory panel of the World Health Organisation recommended measure for pain (Ehrlich, Khalteav 2003).

All experimentally induced pain assessments were performed before and after the first stimulation session, as well as after the final stimulation intervention

session (i.e. total time points = 3). There were no defined rest periods between the different experimental pain assessments. Baseline (i.e. pre-tDCS) experimentally induced pain assessments were measured only at time point 1. Practice of the detection and pain threshold testing for electrical and mechanical stimuli was also incorporated on day 1. All the measurements were performed between 9am and 9pm. The experimental procedure is shown diagrammatically in Figure 4.

Prior to any threshold testing on day 1, an assessment of mechano-receptor sensory function on the participant's hands at three different anatomical locations (i.e. index finger and thenar and hypo-thenar eminences) was briefly performed (see Appendix 8). The participant was blindfolded and asked to verbally indicate 1) whether they perceived either a soft (i.e. piece of cotton) or sharp object (i.e. needle), 2) what hand they perceived the object contacted and 3) whether there was a significant difference in the intensity of the stimuli between hands.

3.11.6.1.5 Self-reported measure of participant blinding awareness

To analyse participant blinding awareness the participant was first asked to answer either yes or no in response to the question "do you believe that you just received the real scalp stimulation intervention protocol?". To further analyse participant blinding awareness, a visual analogue scale (VAS) with a single line and scoring range from 0 for 'not confident at all' to 100mm for 'completely confident' was used (see Appendix 9). The subject was additionally asked to place a vertical mark on the line to indicate the level of confidence they had in their answer to "do you believe that you have just received the 'real' scalp stimulation intervention protocol?". The self-reported

measure of participant blinding awareness has previously been used (O'Connell et al. 2012).

3.11.6.1.6 Self-reported measure of scalp stimulation adverse effects

Completed participants filled in a scalp stimulation adverse effects questionnaire (see Appendix 10). The questionnaire instructed participants to detail the number of occasions a particular adverse effect occurred.

3.11.6.2 tDCS effects on objective measures

3.11.6.2.1 Salivary hormone and neuropeptide

Participants were asked to provide a saliva sample (~1-2 mL) before the stimulation intervention on day 1 and again after stimulation intervention on day 5 (i.e. number of saliva samples = 2). Subjects were instructed not to consume any food or drink, nor brush their teeth for 1 hr before research participation. To minimize any possible effect of diurnal variation, stimulation intervention sessions 1 and 5 were conducted at a similar time for each subject. Subjects were first instructed to allow saliva to pool in their mouth. Subjects were then instructed to tilt their head forward and drool down a piece of plastic straw into a collecting tube. The subject repeated this as often as necessary till sufficient sample was collected. The participant was allowed to physically pretend chewing food (i.e. no physical stimulant was placed in the mouth) to help produce a sufficient amount of saliva. All saliva samples were stored on ice until handling, at which point the samples were aliquotted. All samples were stored at –80°C until use. Upon thawing, the samples were centrifuged to ensure debris removal.

Human cortisol was detected by competitive enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (1-3002; Salimetrics).

Human substance P was detected by competitive ELISA according to manufacturer's instructions (133029; Abcam). Most samples were tested twice, and the mean for those samples were calculated.

Pre intervention saliva samples were collected ~45 minutes prior to stimulation intervention on day 1. Post intervention saliva samples were collected ~ 30 minutes post stimulation intervention on day 5 (refer to Figure 4). Saliva samples were collected approximately between 9:30 am to 7:30 pm.

3.11.6.3 Correlations between baseline psychophysical threshold, subjective and objective measures

All abovementioned psychophysical, subjective and objective measures were used for this study objective.

3.11.6.3.1 Self-reported measure of general pain sensitivity

A self-reported general pain sensitivity questionnaire (SRGPSQ) was also used to add more thorough information regarding the participant's perception of pain sensitivity in relation to imagined experiences of pain (see Appendix 11). The subject was asked to imagine themselves in certain situations and determine whether the situations would be painful. If the situation would be painful, the participant was then asked to state the situational pain intensity on a scale from 1-10 where 0 was 'no pain'; and 10 'the most severe pain that you can imagine.' The administered self-report general pain questionnaire has been previously been shown to significantly correlate to experimental pain intensity ratings (Ruscheweyh et al. 2009).

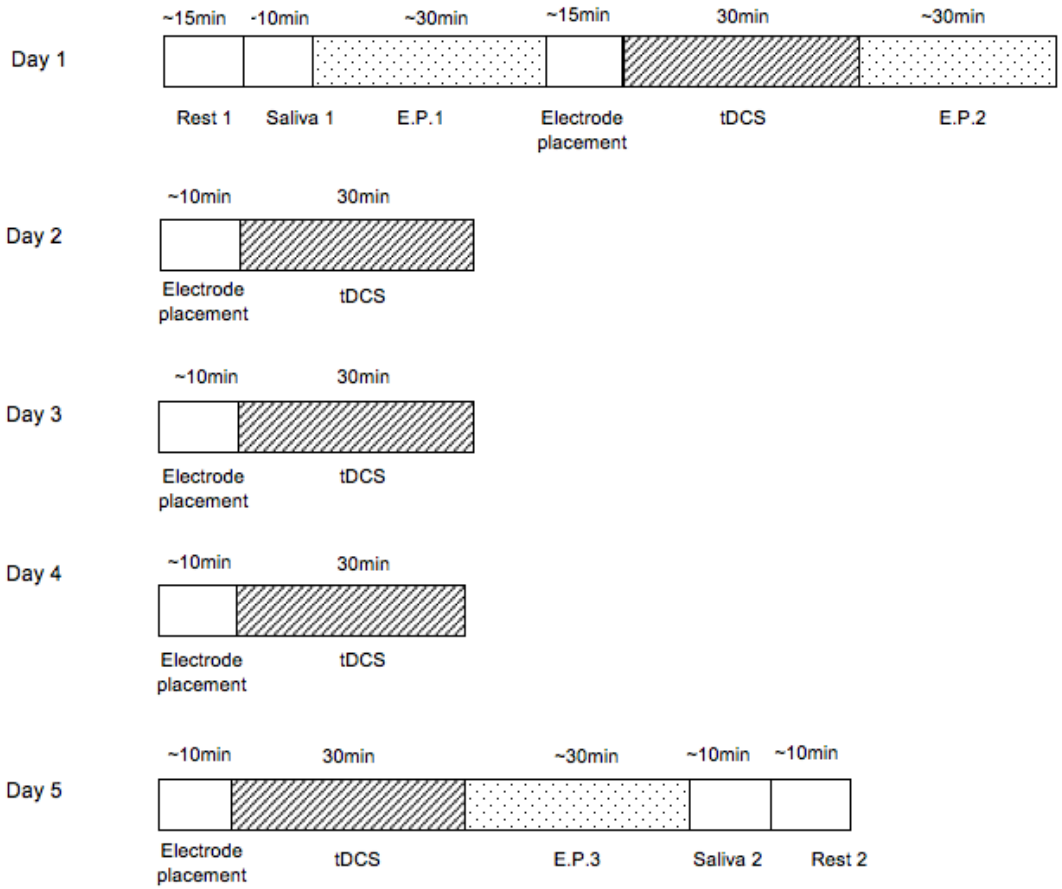

Figure 4 Study design, showing the time course of tDCS treatments and experimental pain measurements. tDCS treatments (30 mins) were delivered once per day for 5 consecutive days. Experimental pain (E.P) measurements were measured before and after tDCS on days 1 and after tDCS on day 5. Baseline (i.e. pre-tDCS) experimental pain measurements were measured at time point 1 only.

3.11.7 Data Analysis

3.11.7.1 tDCS effects on psychophysical threshold and subjective VAS measures

Pooled baseline psychophysical and subjective pain VAS overall, gender based and treatment based means were produced in order to compare means with previous literature.

An independent samples t-test was used to test possible between tDCS group differences in the mean participant blinding visual analogue scale score in order to establish whether the blinding strategy was effective.

The primary analysis endpoint (i.e. primary outcome measure) was the pain visual analogue scales scores, as the visual analogue scale is the expert advisory panel of the World Health Organisation recommended measure for pain (Ehrlich, Khalteav 2003). The psychophysical thresholds were secondary analysis endpoints (i.e. secondary outcome measures).

A mixed model analysis of variance (ANOVA) statistical test analysis was again chosen to test repeated psychophysical threshold/subjective VAS measures at three time points in response to one of two interventions (active or sham tDCS). Refer to section 3.3.6 for the mixed model ANOVA statistcal approach/procedure.

Percentage change from baseline was again also assessed and a mean adverse effects occasion number for each intervention was calculated.

A p-value of ≤0.05 was considered significant for significance tests. For each analysis, IBM SPSS 20.0 for Mac was used.

3.11.7.2 tDCS effects on objective measures

Descriptive statistical analyses were performed (i.e. examining Shapiro-Wilk and visually through histograms) to establish that the raw data met assumptions of normal distribution and normality for statistical tests.

Paired t-test analyses were used to assess within-group differences in the mean concentrations collected pre and post tDCS for cortisol for each tDCS group (active or sham).

Within-group differences were also assessed with Cohen's d effect size measure where d =0.20 is considered a small effect, d=0.50 a medium effect, and d=0.80-infinity a large effect size (Cohen 1992).

Statistical comparisons of mean baseline and change from baseline salivary cortisol concentration between the groups (active – sham) were conducted using an independent t-test.

Statistical comparison of the mean saliva sample 24hr time for each time point and change from baseline between the tDCS groups (active – sham) was conducted using non-parametric Mann-Whitney U test.

Percentage change from baseline was also assessed.

A p-value of ≤0.05 was considered significant and 95% confidence intervals are given for significance tests. For each analysis, IBM SPSS 20.0 for Windows was used.

3.11.7.3 Correlations between baseline psychophysical threshold, subjective and objective measures

Correlations between psychophysical, subjective and objective measures were analysed using bi-variate Spearman's Rho correlation coefficients (see Table 20). Correlation coefficients have previously been used to assess associations between multiple pain measures (Bhalang et al. 2005, Ruscheweyh et al. 2012).

The strength of the correlation coefficient was valued according to (Domholdt 2000) who suggested the following scale: .00-.25 = little, if any correlation; .26-.49 = low correlation; .50-.69 = moderate correlation; .70-.89 = high correlation; and .90-1.00 = very high correlation.

A p-value of ≤0.05 was considered significant for significance tests. It is important to note that the approach to analysing this data was exploratory. Hence, p-value adjustment for multiple comparisons was not performed.

3.12 Results

3.12.1 tDCS effects on psychophysical threshold and subjective VAS measures

Pooled baseline psychophysical and subjective pain VAS overall, gender based and treatment based means were produced (refer to Table 16).

An independent samples t-test displayed that there were no statistically significant between-groups differences in participant blinding VAS scores (active {mean, SD} = 6.35, 2.64; sham {mean, SD} = 7.66,1.52), $t(39)= -1.847$, p = .072). In addition, 23/23 and 17/18 participants answered yes to the

participant blinding awareness question following active or sham tDCS respectively. Hence, the blinding strategy was deemed effective.

With respect to descriptive analyses, CPT and PPT had to be logarithmically transformed in order to meet normality assumptions for parametric testing (refer to Table 17).

CTT and EDT were not analysed as transformations failed to meet normality assumptions for parametric testing. There are two main reasons for this. Firstly, there were a number of values on the natural limit for CTT. Secondly, there was more than one mode (roughly speaking) for EDT.

Table 16 Pooled baseline psychophysical and subjective pain VAS overall, gender based and treatment based means (numbers in brackets represent standard deviations)

	CPT_1 (s)	CPT_V_ 1 (cm)	CTT_1 (s)	CTT_V_ 1 (cm)	EDT_1 (mA)	EPT_V_ 1 (cm)	EPT_1 (mA)	PPT_1 (kPa)	PPT_V_1 (cm)
Overall	11.81	3.48	50.00	7.14	0.91	3.66	1.78	180.77	3.54
	(9.65)	(1.98)	(39.48)	(1.77)	(0.4)	(2.14)	(0.68)	(91.03)	(2.04)
Male	15.79	3.41	58.14	7.11	1.06	4.11	1.81	235.71	3.95
	(15.0)	(1.82)	(42.0)	(1.68)	(0.34)	(1.86)	(0.53)	(119.47)	(1.73)
Female	9.82	3.51	45.93	7.16	0.84	3.46	1.76	152.28	3.33
	(4.53)	(2.09)	(38.28)	(1.85)	(0.42)	(2.25)	(0.74)	(56.06)	(2.18)
Real	13.79	3.48	58.71	7.08	1.00	3.74	1.84	204.57	3.70
	(11.58)	(2.24)	(43.55)	(1.83)	(0.43)	(2.28)	(0.62)	(103.36)	(2.02)
Sham	9.17	3.48	38.39	7.23	0.80	3.54	1.70	150.35	3.33
	(5.47)	(1.63)	(30.7)	(1.74)	(0.34)	(2.00)	(0.75)	(62.65)	(2.09)

Table 17 The effects of tDCS on experimental pain and pain tolerance thresholds

Parameters	Measure Psycho-Physical	Test	F	Sig.	η_p^2	Measure Subjective	Test	F	Sig.	η_p^2
Electric	EDT									
	EPT	Group	.16	.69	.005	EPT_VAS	Group	.004	.95	<. 001
		Time	.17	.81	.005		Time	2.8	.09	.083
		Time x group	.63	.51	.002		Time x group	.38	.61	.012
Mechanical	PPT[a]	Group	.009	.93	< .001	PPT_VAS	Group	.30	.59	.008
		Time	.44	.51	.013		Time	1.7	.20	.045
		Time x group	1.3	.25	.039		Time x group	1.9	.17	.050
Thermal	CPT[a]	Group	4.8	**.03**	.113	CPT_VAS	Group	.15	.70	.004
		Time	.21	.79	.005		Time	.37	.69	.009
		Time x group	.36	.67	.009		Time x group	1.6	.20	.040
	CTT					CTT_VAS	Group	.26	.61	.007
							Time	2.1	.13	.051
							Time x group	.20	.82	.005

[a] = logarithmically transformed
Bold text p<0.05

When comparing mean psychophysical thresholds and subjective pain VAS scores between the tDCS groups (active – sham) at all the time points the ANOVA demonstrated statistically significant between-groups differences for one transformed psychophysical threshold variable (i.e. CPT) ($p = .03$). The group mean estimates indicated that the active group had higher transformed CPT compared to the sham group. The partial eta-squared effect size was medium for the group effect for CPT. However, the ANOVA did not demonstrate statistically significant with-in group (i.e. factor = time) differences as well as no significant time x treatment interaction.

In this study statistically significant group differences at baseline were found for PPT. Therefore, PPT was analysed using baseline data as a co-variate to adjust for baseline differences.

The overall range of the pooled mean percentage change from baseline psychophysical values at time points 2 and 3 is listed below:

- Time point 2:
    - Anodal; -7.2 to +10.5
    - Sham; -5.4 to +8.5

- Time point 3:
    - Anodal; -8.9 to +3.2
    - Sham; -7.3 to -0.7

The overall range of the pooled mean percentage change from baseline subjective values at time points 2 and 3 is listed below:

- Time point 2:
    - Anodal; +0.4 to +15.3
    - Sham; -18.5 to +12.2

- Time point 3:
    - Anodal; -20.6 to +3.5
    - Sham; -10.5 to +2.1

The scalp stimulation adverse effects questionnaire demonstrated that participants in both groups experienced a range of adverse effects (see Table 18).

Table 18 Pooled scalp stimulation adverse effect occasion number treatment based means (numbers in brackets represent standard deviation)

Group	Itching	Tingling	Burning	Pain	Visual	Headache	Skin irritation	Nausea
Active	3.12 (2.18)	2.82 (2.43)	2.59 (2.53)	1.24 (2.17)	0.29 (0.85)	0.65 (1.37)	0.35 (1.22)	0.35 (1.22)
Sham	2.67 (2.50)	3.78 (2.13)	3.11 (2.37)	2.06 (2.46)	0.33 (1.03)	0.28 (0.57)	0.44 (1.34)	0.00 (0.00)

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3.12.2 tDCS effects on objective measures

One participant's data was excluded from data analysis, as samples provided were not collected at a similar time of the day. A pH indicator revealed that another participant's sample was potentially acidic, which can produce artificial results. Hence, a total of 39 saliva samples collected at baseline (i.e. pre tDCS) and time point 2 (i.e. post tDCS) were analysed.

Mean cortisol concentrations had to be transformed (i.e. logarithmically) in order to meet the normality assumption. However, saliva sample 24 hr time could not be transformed in order to meet the assumption of normality.

Table 19 The effects of tDCS on cortisol

	1. Active tDCS			**2. Sham tDCS**				
	Non-transformed mean change from baseline (SD)	Change from baseline p-value (95% CI)	Effect size†	Non-transformed mean change from baseline (SD)	Change from baseline p-value (95% CI)	Effect size†	Non-transformed between group (**1-2**) change from baseline mean difference	Independent samples t-test p-value (95% CI)
Cortisol		(n=22)			(n=17)			
Time point 2	-0.14 (0.2)	**<.001** (.172 to .463)	0.85	-0.07 (0.1)	**.039** (.008 to .262)	0.56	-.076	.063 (-.376 to .011)

† Cohen's *d*, **Bold text p<0.05**

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Statistically significant differences in the mean concentration collected at baseline and time point 2 for cortisol were observed for both active (p = <.001) and sham (p = .039) groups (refer to Table 19). Paired differences revealing a lower mean saliva cortisol concentration at time point 2 for both groups. Cohen's d values for cortisol displayed large and medium effect for active and sham tDCS respectively.

Independent samples t-test analyses displayed that there were no statistically significant between-group differences for cortisol in the baseline mean concentration and mean concentration change from baseline (p = .063).

Mann Whitney U test analyses also displayed that there were no statistically significant between-group differences in the mean saliva sampling 24hr time for each time point (pre: *U* = 153, p = .347; post: *U* = 139.5, p = .181) or change from baseline (*U* = 167, p = .585).

Percentage change from baseline values in this study were ~ -30 and -50 % following sham and active tDCS respectively.

With respect to salivary substance P, the concentration results were hardly ever above the lower limit of detection (i.e. only 4 participants per group could be analysed). As a result, they were not analysed.

3.12.3 Correlations between baseline psychophysical threshold, subjective and objective measures

Statistically significant correlations between baseline psychophysical thresholds for EDT, EPT, PPT, CPT and CTT (e.g. CPT_1 & CTT_1) were

observed. Spearman's Rho correlation coefficient values (r) displayed positive low, moderate and high correlations for statistically significant correlations.

Statistically significant correlations between baseline subjective pain VAS scores for EPT, PPT, CPT and CTT (e.g. EPT_V_1 & CPT_V_1) were observed. Spearman's Rho correlation coefficient values (r) displayed positive moderate and high correlations for statistically significant correlations.

Statistically significant correlations between baseline psychophysical thresholds and baseline subjective pain VAS scores were observed for EDT & CPT_V and CTT & CTT_V. Spearman's Rho correlation co-efficient values (r) displayed either negative moderate or positive low correlations for statistically significant correlations.

Correlations between SRGPSQ scores and all psychophysical, objective and other subjective assessments were not statistically significant.

As well, correlations between baseline salivary cortisol levels and all psychophysical and subjective assessments were not statistically significant.

Table 20 Spearman's Rho correlation analysis of baseline psychophysical thresholds, subjective and objective assessments

	CPT	CPT-VAS	CTT	CTT-VAS	EDT	EPT-VAS	EPT	PPT	PPT-VAS	SRGPSQ	Cortisol
CPT											
CPT-VAS	.068										
CTT	.564[a]	-.224									
CTT-VAS	-.236	.596[a]	-.502[a]								
EDT	.262	.314[b]	.216	.094							
EPT-VAS	.140	.746[a]	-.107	.618[a]	.303						
EPT	.252	.175	.177	.092	.749[a]	.122					
PPT	.141	-.030	.263	.000	.387[b]	.101	.333[b]				
PPT-VAS	.116	.578[a]	-.054	.571[a]	.286	.821[a]	.122	.235			
SRGPSQ	-.075	-.183	.187	-.063	-.133	-.184	-.002	-.244	-.131		
Cortisol	-.032	.058	.080	-.131	-.236	.006	-.280	.086	.013	.078	

[a] Correlation is significant at the 0.01 level (2-tailed).

[b] Correlation is significant at the 0.05 level (2-tailed).

00-.25 = little, if any correlation; .26-.49 = low correlation; .50-.69 = moderate correlation; .70-.89 = high correlation; and .90-1.00 = very high correlation.

3.13 Discussion & Conclusions

3.13.1 tDCS effects on psychophysical threshold and subjective VAS measures

With respect to mean baseline psychophysical thresholds, direct comparisons of thresholds described here and those reported in previous research using psychophysical sensory thresholds are difficult due to methodological differences (e.g. stimulation site, frequency and application rate) (see Table 16). The mean overall EDT (i.e. ~0.9mA) & EPT (i.e. ~1.8mA) values described here were distinctly smaller than body side based mean values reported by Laitinen and Eriksson (1985) (i.e. EDT = ~1.5-2mA; EPT=~3.5mA). The gender based mean CPT (i.e. females = ~10s, males = ~16s) values measured in our research were smaller than those obtained by Tashani, Alabas and Johnson (2010) (i.e. females = ~16s, males = ~24s). The mean gender based CTT (i.e. females =~46s, males = 58s) values were also distinctively higher than those reported by Neziri et al. (2011b) (i.e. females =~35s, males =39s). The mean overall PPT values (i.e. ~180 kPa) reported from our study were also distinctly smaller than those obtained by Rolke et al. (2006) (i.e. 400 kPa).

With respect to tDCS effects on psychophysical thresholds, no statistically significant within-subjects (i.e. factor = time) differences were found. In contrast, a statistically significant between-subjects (i.e. factor = treatment) difference was observed for CPT only. The results therefore do not support our hypothesis that the use of consecutively daily sessions of anodal motor cortex tDCS would result in significantly lower electrical detection thresholds,

and higher electrical, thermal and mechanical pain thresholds compared to the effects of sham in a healthy human population.

The significant between-subjects finding for CPT is in agreement with former controlled tDCS studies in healthy volunteers (Grundmann et al. 2011, Hansen et al. 2011, Bachmann et al. 2010, Jürgens et al. 2012, DosSantos et al. 2014). The non-significant between-subjects finding for PPT is also in agreement with existing research on controlled tDCS studies in healthy volunteers (Grundmann et al. 2011, Bachmann et al. 2010, Jürgens et al. 2012, Vaseghi, Zoghi & Jaberzadeh 2015a). In contrast to the present study interaction findings, Boggio et al. (2008) was able to demonstrate that stimulation type can influence tDCS induced effects on EPT over time.

There are a number of possible methodological reasons why a statistical significant between-subjects difference was seen for CPT and not other psychophysical thresholds. Firstly, the stimulus intensity (i.e. related to temperature) was not purposefully altered during CPT. The CPT may therefore preferentially activate afferent C fibres. In contrast, EPT and PPT are none preferential meaning that all afferent fibres (i.e. a-beta, a-delta) are activated sequentially with increasing stimulation intensity. Secondly, CPT had a larger stimulation area (i.e. hand) compared to EPT and PPT (i.e. finger). Thirdly, CPT was always the final psychophysical threshold test (i.e. after either EPT or PPT) in each experimental pain testing block. Hence, we cannot rule out sequence effects. Ultimately, the research suggests the need for further research before task dependent effects can be elucidated.

Exactly how motor cortex based non-invasive brain stimulation may affect pain function in a healthy population is not fully understood. Several mechanisms are proposed. Motor cortex tDCS could alter sensory discriminative components of pain by influencing descending cortico-thalamic pathways. Polania, Paulus and Nitsche (2012) demonstrated that motor cortex anodal tDCS could alter functional coupling between the ipsilateral motor cortex and thalamus in a healthy population. As well, motor cortex tDCS could alter pain related outcome measures by influencing descending opioid-based anti-nociception. Motor cortex tDCS can affect descending opioid-based anti-nociception via activation of several opioid rich central nervous system sites such as the thalamus, peri-aqueductal grey, precuneus and prefrontal cortices (DosSantos et al. 2014). Hence, tDCS may alter pain function in a healthy population via influencing a range of pain related nervous system regions.

With respect to percentage change, percentage change from baseline values in this study for psychophysical thresholds were mostly minimal (i.e. typically less than or equal to 10 percent), which is in line with the findings of our systematic review (refer to Chapter 2).

With respect to mean baseline subjective thresholds, the mean baseline gender based CTT_V values (i.e. males and females = ~7cm) described here were similar to those obtained by Mitchell, MacDonald and Brodie (2004) (males =~7.5; females = ~8cm). The mean baseline treatment based CPT_V values (i.e. active and sham tDCS = ~3.5cm) described here were smaller than cold pressor test VAS values obtained by Hamner et al. (2014) (i.e. active and sham tDCS = ~6.5cm). However, Hamner et al. (2014) VAS values

represented the 30 second averaged pain VAS score. The mean baseline treatment based EPT_V values (i.e. active and sham tDCS = ~3.5cm) were similar to the EPT pain intensity ratings obtained by Hansen et al. (2011). However, Hansen et al. (2011) used numeric rating scales instead of pain visual analogue scales to assess pain intensity.

With respect to tDCS effects on subjective pain intensity measures, no statistically significant between-subjects or within-subjects differences were observed for any subjective measure. The results thus do not support our hypothesis that the use of anodal motor cortex tDCS would effectively lower subjective pain intensity ratings compared to the effects of sham in a healthy human population.

Previous literature investigating the effects of tDCS on subjective pain intensity has demonstrated conflicting findings. The non-significant findings are in agreement and disagreement with former controlled tDCS studies in healthy volunteers (Jürgens et al. 2012, Hansen et al. 2011, Terney et al. 2008).

Methodological differences may help to explain the contrasting results. For example, Hamner et al. (2014) recently demonstrated significant tDCS induced reductions in average cold pressor ratings compared to sham when assessing average cold pressor ratings at a water temperature of 14 degrees and not 0 degrees. Hence, it could be suggested that future related studies that investigate the effects of consecutive daily tDCS on pain perception use different variable settings for each examined modality. This would enable stronger conclusions to be made about the effectiveness of consecutive daily

tDCS on experimental pain.

Percentage change from baseline values in this study for subjective ratings were marginally higher compared to those for psychophysical thresholds (i.e. typically greater than 15 percent), which is also in line with the findings of our systematic review and previous research (Aslaksen, Vasylenko & Fagerlund 2014).

With respect to participant blinding, no significant between-group differences in mean participant blinding awareness suggests that the participant blinding strategy was effective. The findings are therefore in contrast to research that has previously demonstrated inadequate blinding of tDCS when delivered at 2mA to the motor cortex (O'Connell et al. 2012). Differences in stimulator devices used and study designs (i.e. parallel vs. cross over) may help to explain finding differences when comparing O'Connell et al. (2012) and the present study. However, the present study results are in line with a number of other studies that have since provided evidence that seems to indicate that participants could not distinguish between active and sham tDCS delivered at 2mA to either the frontal or motor cortices (Ihle et al. 2014, Palm et al. 2013, Russo et al. 2013). Further research into the development of more suitable sham techniques may therefore be required to improve methodological quality.

With respect to adverse effects, the findings are similar to Russo et al. (2013) but different to Kessler et al. (2012). Differences in tDCS parameters (e.g. current intensity, stimulation duration) may account for literature

inconsistencies. Further research into adverse effect occurrences between active and sham tDCS is therefore also required.

3.13.2 Limitations

There are methodological issues that need to be addressed. The study was conducted on predominantly young aged university students. Hence, the results from this study may not necessarily translate to other population age groups. Secondly, the participants were blinded to treatment group but the researcher was not blinded due to limitations in resources to finance equipment or additional personnel. Obviously, there is increased possibility for bias in a single blinded trial compared to a double blinded trial. Thirdly, gender imbalances occurred within the study groups. Consequently, gender based variability may have influenced results. Hence, future related trials should be double blinded and better designed to include gender differences.

3.13.3 Conclusion

Consecutive daily sessions of anodal motor cortex tDCS do not appear to have a cumulative effect on experimental pain perception measures in a healthy population.

3.13.4 tDCS effects on objective measures

With respect to within- and between-groups statistical comparison of saliva concentrations, both anodal and sham motor cortex tDCS significantly reduced levels of salivary cortisol compared to baseline post experimental pain stimulation but there were no significant between-group differences. The results therefore do not agree with our hypothesis that consecutive daily

sessions of anodal motor cortex tDCS would alter salivary levels of cortisol compared to sham post experimental pain stimulation.

The within-group cortisol results (i.e. significance compared to baseline) are in agreement with a prior controlled tDCS study in a healthy population (Raimundo, Uribe & Brasil-Neto 2012). However, the between-group cortisol result (i.e. no significance compared to sham) is in disagreement with prior controlled tDCS studies (Brunoni et al. 2013, Sarkar, Dowker & Kadosh 2014).

Possible explanations for the contrasting results may relate to the site of stimulation or context of the stress response. For example, research has previously demonstrated that a single session of anodal pre-frontal cortex tDCS modulated the effects of arithmetic decision on salivary cortisol compared to sham (Sarkar, Dowker & Kadosh 2014). Further research is therefore required to investigate the influence of stimulation site or context of stress response on tDCS induced effects on cortisol.

Another reason for the contrasting results may relate to power. The results showed a trend toward significant between group differences (i.e. the active tDCS group displayed a considerable larger effect size and smaller level of significance compared to the sham tDCS group). It could therefore be argued that the study was underpowered to achieve between group significance. Perhaps a larger sample size would have yielded group significance.

The lowering in cortisol compared to baseline may therefore reflect a stress response to new procedures; baseline cortisol was measured prior to tDCS and psycho-physical testing whereas follow up cortisol was measured post

tDCS and psycho-physical testing (Raimundo, Uribe & Brasil-Neto 2012). Future studies should therefore have more appropriate timed cortisol measures.

Alternatively, the lowering in cortisol compared to baseline may be due to circadian variation of cortisol levels (Dorn et al. 2007, Baeken et al. 2009). No statistically significant between-group differences in saliva sampling 24hr time for each time point and change from baseline in our study however suggests that circadian variation is not an issue in our study.

With respect to percentage changes, the magnitude and direction of percentage change from baseline cortisol values following anodal and sham tDCS was similar to those previously reported using single session tDCS (Binkofski et al. 2011).

It is also important to discuss how tDCS might have influenced cortisol. Motor cortex tDCS could influence stress related central nervous system circuitry. Cortisol is an end product of the hypothalamic-pituitary-adrenal (HPA) axis. Binkofski et al. (2011) demonstrated that a single session of anodal motor cortex tDCS could lower serum cortisol levels compared to sham tDCS in a healthy human population. The findings therefore suggest that motor cortex tDCS may be able to influence central stress related circuitry such as the hypothalamic-pituitary-adrenal (HPA) axis. Future related studies are therefore required to investigate the relationship between tDCS and cortisol using different mediums (e.g. serum).

With respect to salivary substance P, the concentration results were hardly ever above the lower limit of detection. One possible reason for this may be

that peripheral substance P triggered in peripheral experimental pain testing protocols may not be long lasting enough to influence salivary concentration in many healthy humans.

3.13.5 Limitations

There are methodological issues that should be taken into consideration when interpreting the results. Participants were not asked to refrain from non-work-related vigorous physical activity prior to research participation. Previous research has demonstrated that physical activity can increase objective pain related biomarker levels in the human body (Lind et al. 1996, Lusa Cadore et al. 2009). However, no recent vigorous physical activity before testing was reported by participants or physically evident when observing participants before testing. Furthermore, the study did not control for menstrual cycle phase or use of oral contraceptives, which may have influenced salivary measurements in females (Kirschbaum et al. 1999).

3.13.6 Conclusion

Five consecutive daily sessions of anodal motor cortex tDCS does not significantly alter salivary cortisol levels post experimental pain stimulation compared to sham. It is suggested that future related studies investigate the effects of repeated sessions of anodal pre-frontal tDCS on objective pain related outcome measures.

3.13.7 Correlations between baseline psychophysical threshold, subjective and objective measures

With respect to correlations between psychophysical thresholds, statistically significant correlations between baseline psychophysical thresholds were observed. The results are therefore in line with previous literature that has demonstrated statistically significant correlations between psychophysical measures within the same test modality and between different test modalities in a healthy population (Bhalang et al. 2005, Neddermeyer, Flühr & Lötsch 2008, Neziri et al. 2011a). Bhalang et al. (2005) and Neziri et al. (2011a) further reported higher correlations between psychophysical measures within the same test modality. The highest statistically significant correlation between baseline psychophysical thresholds in the present study was also observed within the same modality (i.e. EDT and EPT) (see Table 20). A higher correlation within the same modality should be expected due to similar characteristics of sensation evoked by psychophysical thresholds within the same modality (Bhalang et al. 2005). Moreover, the strength and direction of correlations between psychophysical measures with in the same test modality reported by Bhalang et al. (2005) are comparable with those described in the present study.

The present study also provides evidence for significant correlations between baseline subjective VAS assessments within and between modalities, as well as between baseline subjective VAS assessments and psychophysical thresholds within and between modalities (see Table 20). These findings therefore agree and disagree with previous literature. Ruscheweyh et al. (2010) demonstrated significant correlations between subjective

assessments, as well as between subjective assessments and psychophysical thresholds within the same modality. Ruscheweyh et al. (2010), however, did not demonstrate significant correlations between subjective assessments and psychophysical thresholds between modalities. It may be difficult to directly compare results to those reported by Ruscheweyh et al. (2010) as the present study incorporated a pain visual analogue scale for subjective assessment compared to a numeric rating scale utilised by Ruscheweyh et al. (2010).

The current study further found that SRGPSQ scores did not significantly correlate with any baseline psychophysical, subjective or objective assessment. The finding that SRGPSQ scores did not significantly correlate with baseline psychophysical threshold is in line with previous literature conducted in a healthy population (Ruscheweyh et al. 2009). Ruscheweyh et al. (2009), however, reported that SRGPSQ scores significantly correlated with subjective assessments. Again, it may be difficult to directly compare results to those reported by Ruscheweyh et al. (2009) as the present study incorporated a pain visual analogue scale for subjective assessment compared to a numeric rating scale utilised by Ruscheweyh et al. (2009).

In summary, the present study provides evidence that baseline salivary cortisol levels did not significantly correlate with any psychophysical threshold or subjective assessment in a healthy population. These findings are in agreement with previous literature that demonstrated no significant correlations between thermal (i.e. heat) pain and hormone (i.e. cortisol/ dehydroepiandrosterone) plasma levels in healthy women (Yamamotová,

Kmoch & Papežová 2012). It therefore appears that salivary/peripheral levels of cortisol would not help clinicians interpret pain intensity.

3.13.8 Limitations

The study was exploratory. Hence, p-value adjustment for multiple comparisons was not performed.

3.13.9 Conclusions

Statistically significant correlations between psychophysical thresholds, between subjective assessments and between psychophysical thresholds and subjective assessments were observed in a healthy population. The observed correlations may suggest the clinical utility of different types of pain assessments.

Chapter 4

General Discussion & Conclusion

4.1 Research objectives, key findings and potential explanations

Numerous studies have investigated the effects of transcranial direct current stimulation (tDCS) on measures of somatosensory perception in a healthy population. However, the systematic review undertaken within this thesis and two previous systematic reviews (Vaseghi, Zoghi & Jaberzadeh 2014, Vaseghi, Zoghi & Jaberzadeh 2015b) indicated both methodological limitations and heterogenous tDCS induced effects for existing trials. These three reviews also reported that stimulation frequency (e.g. using repeated daily tDCS) was one area that researchers have failed so far to focus their attempts on.

The overall primary purpose of Studies 1 and 2 was therefore to investigate the effects of consecutive daily sessions of anodal tDCS on psychophysical, subjective and objective outcome measures in a healthy human population. Following this, a secondary purpose of Study 2 was to explore correlations between baseline psychophysical, subjective and objective outcome measures in a healthy population.

The overall primary finding from Studies 1 and 2 suggests that increasing stimulation frequency (e.g. using repeated daily tDCS) does not appear to have a cumulative effect on psychophysical (i.e. vibration, electrical, pressure and thermal detection and pain thresholds), subjective (electrical, pressure

and thermal pain visual analogue scales) and objective (cortisol) measures in a healthy human population.

The results predominantly do not support our hypothesis that five consecutive daily sessions of anodal tDCS would significantly lower electrical, vibration detection thresholds, heighten electrical, thermal and mechanical pain thresholds and lower subjective pain scores (i.e. electrical, thermal and mechanical pain visual analogue scales) compared to the effects of sham tDCS in a healthy human population.

There are a number of potential reasons for this that relate predominantly to methodology and experimental design. With respect to methods, there are certain intervention related limitations such as focality, inter-individual variability and strength. As mentioned in the introduction chapter, one tDCS limitation is stimulation focality with conventional tDCS potentially producing nerve polarization over a large area of brain. Combining tDCS with other techniques, which promote activity in related nervous system areas, may have therefore been required to achieve more consistent results by improving the specificity of tDCS induced neuroplasticity (Cano et al. 2013). In line with this hypothesis, Schabrun et al. (2014) demonstrated that combining motor cortex tDCS and peripheral electrical stimulation (i.e. applied to the body area of most pain) more effectively improved chronic low back pain symptoms in a chronic pain population compared to either technique alone or sham tDCS.

Another key limitation of tDCS is high inter-individual variability in both neurophysiological and behavioural responses (Wiethoff, Hamada & Rothwell 2014). A number of factors that can modify plasticity induction have been

identified, such as age, sex and handedness (Ridding, Ziemann 2010). It is therefore plausible to suggest that significant treatment effects may have been obtained if we focused on only the one gender or a different aged population. Of importance to this thesis, previous research has demonstrated that baseline brain metabolite levels predicted the tDCS induced analgesic response in a healthy population (Reidler et al. 2012). Consequently, it could be hypothesised that investigating tDCS induced effects on experimental pain in populations with certain biochemical profiles may also help to achieve more consistent results. The abovementioned research highlights the need to better understand the individual factors that determine tDCS responsiveness.

Thirdly, there are no standard protocols/markers to assess effectiveness of tDCS strength (or dose) (Priori, Hallett & Rothwell 2009). tDCS strength is therefore not individually adjusted. Consequently, it could be hypothesised that the use of more individualised tDCS strength parameters may help to achieve more consistent results.

The outcome measures used in this study may have also been another methodological factor that influenced findings. Most outcome measures in this study were pain related. Pain can be viewed as a dynamic process that can be influenced by a number of individual factors. The evaluation of pain can therefore be considered as being more complex compared to other physiological measures such as evoked potentials, for which tDCS has been shown to consistently influence in a healthy population (Ihle et al. 2014, Nitsche et al. 2008).

As well, the research did not investigate tDCS induced effects on serum/plasma levels of cortisol or substance P. Previous research has demonstrated less variation in serum levels of cortisol compared to saliva (Reynolds et al. 1998). Additionally investigating potential effects on serum/plasma levels could have allowed for stronger conclusions to be made about the effectiveness of consecutive daily sessions of tDCS on pain related biomarkers.

With respect to experimental design, there are a number of factors that may have shaped the findings such as intervention timing, population type and outcome measure settings and timing.

Firstly, it may be that the stimulation timing (i.e. once daily) was sub-optimal to elicit cumulative longer lasting effects on the sensory domain in a healthy population. Indeed, the results of a recent systematic review suggested a lack of effect for current multiple dose tDCS strategies (e.g. once daily) on pain related outcome measures in a chronic pain population (O'Connell et al. 2014).

One alternative approach may be to deliver successive tDCS using shorter intervals (i.e. in the order of minutes) (Goldsworthy, Pitcher & Ridding 2014). In support of this approach, Monte-Silva et al. (2013) demonstrated that continuous application of 1mA motor cortex tDCS for 26 minutes resulted in motor pathway excitability changes that were shorter in duration when compared to spaced stimulation of the same total duration (i.e. 2x13 min of tDCS with 3 or 20 min interval) in a healthy population. In contrast, spaced stimulation of the same total duration using inter-tDCS intervals of hours

abolished plasticity induction. Similarly, research has demonstrated that within session repeated tDCS (i.e. two or three applications of 10 minute tDCS with an interval of 25 minutes) was preferable for modifying motor pathway excitability and motor behavior (e.g. Purdue pegboard test) in a healthy population compared to a single application of 10 minute tDCS (Bastani, Jaberzadeh 2014). Further research into the effect of tDCS timing on psychophysical, subjective and objective measures is therefore warranted.

Secondly, using healthy, pain free individuals to study pain mechanisms allowed for enhanced experimental control (i.e. related to stimulus intensity, frequency, localisation and duration) compared to clinical pain studies (Staahl, Drewes 2004). However, the short lasting stimuli used in this study does not reproduce actual clinical pain (Staahl, Drewes 2004). Consequently, the negative findings from this study may therefore not translate to a persistent pain population.

As well, there was an attempt made to examine the effects of tDCS on substance P. However, the concentration results were hardly ever above the lower limit of detection. As significantly higher levels of salivary substance P have been found in chronic pain patients compared to healthy controls, it could be suggested that future related studies should focus on clinical chronic pain populations to evaluate the potential effects of consecutive daily sessions of tDCS on objective pain related outcome measures (Jang et al. 2011).

Thirdly, another reason for the findings may be due to outcome measure settings and timings. Firstly, different variable settings were generally not used for each examined modality. For example, psychophysical thresholds in

Study 2 were only assessed on the body side contralateral to tDCS. However, change in a psychophysical threshold (i.e. innocuous cold sensitivity) on the body side ipsilateral to tDCS has previously been reported (Bachmann et al. 2010). Furthermore, CPT was only assessed at the one temperature. Interestingly, Hamner et al. (2014) recently demonstrated significant tDCS induced reductions in CPT subjective ratings compared to sham when assessing CPT at a water temperature of 14 degrees and not 0 degrees. Hence, it could be suggested that future related studies that investigate the effects of consecutive daily tDCS on pain perception use different variable settings for each examined modality. This could possibly enable stronger conclusions to be made about the effectiveness of consecutive daily tDCS on experimental pain.

With respect to outcome measure timing, the participant performed several psychophysical measurements with the same standardised procedure. However, the repeated sessions design can be susceptible to test-retest bias (e.g. retest performances influenced by previous sessions). Test-retest bias with psychophysical measures has previously been reported (Teepker et al. 2010). In Study 1, it can be seen that for both active and sham tDCS groups that there was a steady reduction in 30Hz VDT for both sides over time. These findings could indicate that a learning or training effect may have been present for 30Hz VDT. Factoring session-to-session effects into the analyses would have required repeated psychophysical tests before start of the trial. This would have required more resources (i.e. project finances, participant time) to do so. Nonetheless, future related studies should factor session-to-session effects to minimise the possibility of test-retest bias.

Moreover, we did not investigate the long term effects of consecutive daily sessions of tDCS induced effects. It is therefore not known if tDCS may have induced a long-term effect (Lang et al. 2007). Future related studies therefore should incorporate measures that can investigate potential long term tDCS induced effects.

With respect to the correlations, the thesis demonstrated statistically significant correlations between psychophysical thresholds, between subjective assessments and between psychophysical thresholds and subjective assessments in a healthy population.

There are a number of potential finding implications. To begin with, the results may provide further evidence of the clinical utility of different types of pain assessments. However, the weak to moderate correlations also suggest that different types of body pain stimuli assessments should be used to appropriately evaluate the level of an individual's pain sensitivity. Likewise, caution must be applied in the interpretation of an individual's pain sensitivity based off a single body pain stimuli assessment (Bhalang et al. 2005). It can also be suggested that future studies investigate whether the level of an individual's pain sensitivity assessed using a number of different body pain stimuli can indicate persistent pain condition development (Bhalang et al. 2005).

4.2 Concluding remarks

Advancements in neuroscience methods have subsequently led to the use of non-invasive brain stimulation techniques in research. The challenge that is

consequently posed is can these techniques effectively harness the power of neuroplasticity for distinct and meaningful purposes, such as to further improve human body function or even treat certain disorders?

This thesis attempts to make a contribution to addressing this research problem. It was hypothesised that the increasing stimulation frequency (i.e. using repeated daily tDCS) would enhance the consistency/efficacy of tDCS induced changes to somatosensory and pain perception in a healthy population. In contrast to this hypothesis, this was the first study to demonstrate that a once daily multiple dose strategy was sub-optimal to produce consistent and stronger anodal tDCS induced changes to somatosensory and pain perception in a healthy population. These findings may therefore help direct future research to develop more appropriate dose strategies that may eventually lead to therapeutic use of tDCS in the treatment of nervous system disorders such as in pain and stroke conditions.

In addition, the research has provided further evidence that significant correlations exist between psychophysical thresholds, between subjective assessments and between psychophysical thresholds and subjective assessments in a healthy population. With further research, these findings may help lead to more appropriate evaluations of pain sensitivity in clinical populations.

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Appendices

Appendix 1 Study 1 explanatory statement

BOND UNIVERSITY
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CRICOS CODE 000017B

Explanatory Statement

Bond University Human Research Ethics Committee Protocol Number: 1439

Study title: Modulation of somato-sensory cortex function with non-invasive brain stimulation.

Investigators:

Supervisor: Associate Professor Peter Johnson

PhD Student: Brookes Folmli

Research Aims

Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are both non-invasive techniques capable of activating elements of the brain without causing pain. Recent research has provided evidence for the ability of both TMS and tDCS in inducing short term changes to certain regions within the human brain, which outlast the stimulation period. Effectively utilising non-invasive brain stimulation to temporally alter brain function could therefore improve our understanding of the human sensory system.

Although research has further shown both TMS and tDCS to have effects on behavioural aspects of sensory function in healthy populations, more research is overtly required to advance our understanding of the potential effects on these measures with various repetitive TMS (rTMS) and tDCS based stimulating paradigms and protocols. In particular, no research has yet compared the effects of separate repeated session protocols of either TMS or tDCS on behavioural features of sensory processing.

Vibration detection thresholds represent an objective method of measuring human sensory function in research procedures. It could therefore be hypothesized that the use of both protocol types would effectively modulate vibration detection thresholds. Consequently, the

aim of the current research project is to investigate the potential of different non-invasive brain stimulation protocols, namely TMS and tDCS, in changing specific measurable behavioural aspects of sensory function. At the same time, evaluating differences between the two protocols. Equipped with this knowledge, better directed clinical and pharmacological investigations related to the area could potentially be produced

Procedures

You will first be asked to read through the list of risks with the use of both TMS and tDCS (please see "List of risks with the use of TMS & tDCS" on page 5). You will then be asked to answer the pre-study questionnaire. If eligible, you will be subsequently allocated randomly to either a TMS or tDCS protocol group.

During the first, third and final stimulation sessions only, vibration detection thresholds will be performed. An initial examination of vibration detection thresholds will be conducted. Vibrations will be delivered specifically to the skin surface of two different upper limb locations (i.e. finger on both sides) at two different frequencies via a mechanical vibrator. Vibration detection thresholds will involve placing your finger on the vibrator's probe tip, which will be protruding through a hole in a perspex plate encasing it. Throughout the experiment, a series of different vibration amplitudes will be delivered to the skin surface, for which you will be asked to state whether or not you can detect the stimulus. For each site and frequency, the mean of 10 detection thresholds will be calculated.

If you are in the TMS group, your level of motor cortex excitability will be measured first (i.e. prior to the pre repetitive stimulation vibration measurement). For this, self-adhesive surface electromyography (EMG) electrodes will be attached to your hand muscles to record the bioelectrical activity of these muscles in response to TMS. In order to evoke a muscle activity, stimulation of the motor cortex will be performed using a circular shaped coil. Your active motor threshold will be established, which is the lowest intensity required to consistently produce muscle activity in the contra-lateral hand to cortical stimulation during slight contraction of the hand muscles. Your active threshold will determine whether you are allowed to continue in the TMS group. If you are not allowed to continue in the TMS group, you will be placed in the tDCS group. If allowed to continue, the site of stimulation will then be located. Subsequent to the pre repetitive stimulation vibration measurement, rTMS will be applied at an intensity 20 % below that of your active threshold.

If you are not in the TMS group, tDCS will instead then be delivered at a current intensity of 1mA over the site of stimulation. For both groups, the vibration detection threshold will then be measured again post-stimulation to be compared with the before stimulation measurements. This session format will be repeated again on your third and final sessions. Sessions 2 and 4 will involve stimulation only. The experiment will therefore require you to participate for ~7hrs over five separate sessions on 5 consecutive days. Your time commitment will be compensated, i.e. 2x lunch vouchers ($40/ voucher) or $100.

Risks and Discomfort

Currently, there are some known and potential risks associated with the use of both TMS and tDCS (please see “List of risks with the use of TMS & tDCS” on page 5). One possible adverse effect associated with TMS is the rare induction of a seizure. Research that has used the type of TMS protocol that will be applied in this study (i.e. 67 studies - number of participants totaled 1040 persons) has so far only reported one case of an induced seizure. In line with these findings, the risk for seizure induction is therefore considered very low. During or after stimulation muscle tension, headache or neck pain may arise due to activation of scalp and neck muscles, but these respond promptly to common analgesics. In sensitive subjects, a tingling sensation and twitch of facial muscles may also be felt during the application of TMS. The only absolute contra-indication to TMS is the presence of metallic hardware that is in close contact with the coil. Therefore, persons with metal implants will not be invited to participate. All procedures performed with TMS will strictly adhere to the measured safety guidelines.

A direct current stimulator is operated by a common household battery and during stimulation there is hardly a sensation felt over the area of contact. Occasionally, headache, dizziness, nausea, fatigue, visual sensation, skin irritation and a tingling sensation under the area of the electrodes are reported as side effects. tDCS will also be performed with procedures similar to those which have been previously considered safe in humans. The PhD student will also go through the full list of risks associated with TMS and tDCS with you during your initial consultation before you give your consent.

Confidentiality

Confidentiality of your records will be adhered to. Your results will be securely stored on computer files, for which only the above researchers will have access to. All data corresponding to your results will be number coded so you will not be identified in any future reports or publications.

Volunteer Participation

You are free to withdraw from the conducted experiment at any stage of the research.

Debriefing

If you would like to be informed of the aggregate research finding, please contact the student investigator. A summary of the findings and how this has contributed to scientific knowledge will be emailed to those wanting this information.

Counseling Services

In addition to research enquires, if you require counselling over any experiences throughout the experiment please call:

Lifeline Australia: 131 114; Bond University Counselling Office: 55 954 002

Research Ethics

If you have any concerns in regards to the conduct or nature of this research (RO1439), please do not hesitate to contact the Bond University Research Ethics Committee at the following address:

Senior Research Ethics Officer
Complaints
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Telephone: (07) 5595 4194 Fax (07) 5595 1120 Email: buhrec@bond.edu.au

Research Inquires

If you have any queries or would like to be informed of the aggregate research finding, please contact:

Student Investigator: Mr. Brookes Folmli

Signature: …….

Mobile: 0432 102 778

Investigator: Associate Professor Peter Johnson

Signature: ……

Telephone: 5595 4048

Address: School of Health Sciences & Medicine, Bond University 4229

List of risks with the use of TMS & tDCS

Known adverse effects with TMS:

1. Seizures: Single pulse TMS has resulted in seizures in patients, but not in normal subjects, whereas repetitive TMS has resulted in seizures in both patients and normal subjects. 16 cases of TMS induced seizures have been reported. Considering the large number of experiments conducted using TMS, the risk of inducing seizures is very low.

2. Heating of the brain: Heating of the brain is unlikely to cause injurious effects. The theoretical power exposed to during TMS is a few milliwatts at 1 Hz; while the brain's metabolic power (13 W).

3. Heating sensation of the scalp: TMS can induce sensations of heat on the scalp due to coil heating; however, there is a monitor on the machine, which alerts when temperatures approach forty degrees Celsius.

4. Syncope (fainting): Syncope can occur as an epiphenomenon (i.e. not related to direct brain effects). A syncope management plan has been implemented.

5. Headaches and Local Pain: During TMS, induction of muscle tension headache or neck pain may arise possibly due to activation of scalp and neck muscles, which respond promptly to an aspirin, acetaminophen (Tylenol ®) or other common analgesics.

6. Subjects may also experience nausea, and twitching of the face.

Potential complications of TMS:

1. Cognition: Repetitive TMS may result in memory problems and other cognitive deficits. These effects are very rare, mild, and very transient. Several safety studies with rTMS have revealed no adverse long-term effects or sustained changes in cognition. Most TMS studies have not seen any effects of rTMS on mental abilities, and some have actually improved cognitive function.

2. Kindling: The process of repetitive sub-convulsive shocks leading to a subsequent epileptic event is highly unlikely. This is due to the fact kindling requires frequencies of at least 60Hz, and a pulse duration of 1ms longer than that seen in TMS.

3. Exposure to Magnetic fields: As TMS magnetic fields are only 2 Tesla and rapidly decaying; this should not have adverse effects.

4. Endocrine function: There have been several reports of changes in hormone levels, in particular changes to pro-lactin levels, thyroid-stimulating hormone. However, there has been no evidence for clinically relevant changes in hormone functions.

5. There have also been several reports of TMS induced changes to immune function, neurotransmitter levels & autonomic function. However, no deleterious effects have been reported.

Known adverse effects with tDCS:

Headaches and local pain, dizziness, nausea, fatigue, skin irritation and a tingling, itching or mild burning sensation under the area of the electrodes have all been reported as side effects with the use of tDCS. A visual sensation, associated with switching 'on' and 'off' the stimulation has also been reported.

Potential complications with tDCS:

1. Electrochemically produced toxic brain products and metallic electrode dissolution products at the electrode-tissue interface: In tDCS, there is no direct contact between the electrodes and brain tissue. As well, the metallic electrode will be placed between two sponges, which should act to buffer the skin from electrochemical changes**.**

2. Current-induced neuronal hyper-excitability and brain tissue heating: This specifically refers to effects induced by high frequency supra-threshold stimulation lasting for hours. Considering tDCS induces only moderate changes in cortical excitability, a damaging effect by neuronal hyper-activity seems improbable.

Appendix 2 Study 1 pre-study questionnaire

Pre-study questionnaire

Please read the following items carefully and put a tick (✓) on the answer that best corresponds to your current situation. Please be advised that the responses you provide for this questionnaire will be kept strictly confidential and will be used only for the purposes of this research. Therefore, please answer as honestly as you can. If you have questions please do not hesitate to ask the investigators: Brookes Folmli (bfolmli@student.bond.edu.au) and Peter Johnson (pejohnso@bond.edu.au).

1. Subject Details	
Name, SID	
Mobile number	
Emergency contact (number, name + relationship to subject)	
Age	
Date form filled	
Gender	Male ☐ Female ☐
Handedness	Right ☐ Left ☐
How did you find out about this research?	

2. Eligibility	
If you tick yes to any of the following questions in Part A, you will be excluded from the study:	
PART A:	
Do you have any metallic or magnetic pieces inside your brain/skull (except titanium) (e.g. splinters, clips)?	Yes ☐ No ☐
Do you have any implanted metal devices that you are aware of (e.g. cochlear, pacemaker, medication pumps, and neuro-stimulators such as deep brain stimulators)?	Yes ☐ No ☐
Do you have epilepsy or have ever experienced a convulsion or seizure?	Yes ☐ No ☐
Do you have any first degree relatives with epilepsy?	Yes ☐ No ☐
Do you have any hearing problems or tinnitus (ringing in the ears)?	Yes ☐ No ☐
Are you someone who consumes heavy amounts of alcohol (e.g. +4 standard drinks/day very regularly)?	Yes ☐ No ☐
Have you had any recent or severe heart disease?	Yes ☐ No ☐
Are you pregnant or is there any chance you might be?	Yes ☐ No ☐
If you tick yes to any of the following questions in Part B, an individual evaluation of your eligibility will be determined:	
PART B:	

Do you have or have ever experienced any focal or generalized neurological condition (e.g. tumour, stroke, encephalitis, severe head trauma, fainting spells)? – if **other** please specify in the available space underneath the boxes	Yes ☐ No☐
Have you had any recent upper limb injuries? If **yes** please specify the type of injury in the available space	Yes ☐ No☐
Have you ever been involved in a transcranial magnetic stimulation (TMS) and/or transcranial direct current stimulation (tDCS) study? If **yes** please specify the type of study in the available space.	Yes ☐ No☐
Do you suffer from any skin condition (e.g. eczema)?	Yes ☐ No☐

Please list any medication you are taking (or have recently withdrawn from) in the space below:

Appendix 3 Study 1 informed consent

BOND UNIVERSITY
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(from overseas)
Email: hsm@bond.edu.au
ABN 88 010 694 121
CRICOS CODE 00017B

Statement of Consent

Bond University Human Research Ethics Committee Protocol Number: 1439

I agree to participate in the above Bond University study. I fully understand that my participation in the study is voluntary only, and can withdraw from the experiment at any time. I also recognise that I will not be identified in any reports on the project, nor to any other party. I have also read the attached explanatory sheet and am fully aware of all associated aims, procedures and risks involved with the investigation. Therefore, I am willing to:

- Provide my age and handedness to be used in future reports and publications in a non-identified form,
- provide a series of vibration detection threshold measurements 6 times over 3 separate days,
- allow the researchers to perform either transcranial magnetic stimulation (TMS) OR transcranial direct current stimulation (tDCS) on me,
- have my results of all assessment items be used in future reports and publications in a non-identified form.

Name (please print): .....

Signature: .....

Date: ……..

Independent witness

I believe that ……

fully understands the above project and gives her/his consent voluntarily.

Name (please print): .....

Signature: .....

Date: ……

Address: School of Health Sciences & Medicine, Bond University 4229

Appendix 4 Study 2 explanatory statement

BOND UNIVERSITY
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Fax: +61 7 5595 4122
(from overseas)
Email: hsm@bond.edu.au

Explanatory Statement

Bond University Human Research Ethics Committee Protocol Number: RO1693

Study title: Modulation of experimental pain with non-invasive brain stimulation.

Investigators:

Supervisor:	Assistant Professor Allan Abbott, Associate Professor Peter Johnson & Professor Wayne Hing
PhD Student:	Brookes Folmli

Research aims and benefits

Recent development of non-invasive brain stimulation techniques has provided important insight into human nervous system function. Transcranial direct current stimulation (tDCS) is one such technique, which involves delivering electrical current to the brain via surface electrodes. Further research, however, is required to evaluate the effects of consecutive daily application of tDCS on human sensory function. Consequently, the aim of the current research project is to investigate whether five consecutive daily sessions (1 session / treatment day) of tDCS can improve sensory function in a healthy human population. The results of this study will lead to better 1) understanding of human sensory function modulation and 2) directed clinical and pharmacological investigations related to the area.

Procedures

You will first be asked to read through the explanatory statement. You will then be asked to answer the ‘pre-study questionnaire.’ If eligible, you will be subsequently allocated randomly to either the ‘real’ or ‘placebo’ tDCS treatment group.

Prior to the stimulation intervention, an assessment of your sensitivity to electrical, thermal and mechanical induced pain (i.e. experimentally induced pain) stimuli will be performed. For this, a series of pressure, thermal and electrical stimuli will be delivered to the skin surface of your dominant hand. You will be asked to state when you can first 'detect' the electrical stimulus. The mean of 3 'detection' thresholds will be calculated. The electrical, thermal and pressure stimuli will be delivered until you can first confidently perceive the stimulus as being 'painful.' For each type of stimulus, the mean of 3 'pain' thresholds will be calculated. Furthermore, the thermal stimuli will continue to be delivered until you can no longer tolerate the stimuli. As well, you will be asked to indicate the intensity of the pain when you first perceived the stimulus as being 'painful' and 'no longer tolerable' on a visual analogue scale.

Once the experimentally induced pain measures have been performed, tDCS will then be delivered over the site of stimulation via two scalp electrodes on 5 consecutive days (1 session / treatment day).

The 'experimentally induced pain' assessments will be performed again after the first and final stimulation intervention sessions. As well, you will be asked to indicate 1) YES or NO to the question ''Do you feel that you have just received the 'real' stimulation intervention?'' and 2) the level of confidence in your answer on a numeric rating scale. You will also be asked to complete an adverse effects questionnaire and questions related to general pain sensitivity during your final session. Additionally you will be asked to provide a saliva sample before and after the stimulation intervention. This will enable the research to determine if tDCS induced effects on sensory function are associated with changes to the saliva level of a particular biological substance, which is known to impact the nervous system. Sessions 2-4 will involve brain stimulation only. The experiment will therefore requires you to participate for ~5.5hrs spread over 5 separate sessions (1 session / day). Your time commitment will be compensated, i.e. $100.

Research Site

The study will be performed at Bond University, Robina.

Risks and Discomfort

Research has reported that 18% of subjects found the stimulation procedure mildly unpleasant. The following **mild discomforts** were reported during stimulation: tingling (70%), itching (30%), non-harmful burning sensation (22%) and local pain (16%) under the area of the electrodes. Some subjects reported mild and short-term headache (4%), nausea (3%), skin irritation (i.e. mild redness), and a visual sensation (i.e. flash of light) (9%) when turning the stimulation on and off. Potential discomforts with experimental pain include short-term local pain and skin irritation during testing.

To minimize this, the stimulation protocols will be adhering to safety guidelines. These potential risks will be lowered further through the use of thorough subject exclusion criteria.

The PhD student will also go through the full list of risks associated with tDCS and experimental pain with you during your initial consultation before you give your consent.

Confidentiality

Confidentiality of your records will be adhered to. Your results will be securely stored on computer files, for which only the above researchers will have access to. All data corresponding to your results will be number coded so you will not be identified in any future reports or publications.

Volunteer Participation

You are free to withdraw from the conducted experiment at any stage of the research.

Debriefing

If you would like to be informed of the aggregate research finding, please contact the student investigator. A summary of the findings and how this has contributed to scientific knowledge will be emailed to those wanting this information.

Counselling Services

In addition to research enquires, if you require counselling over any experiences throughout the experiment please call:

Lifeline Australia: 131 114

Bond University Counselling Office: 55 954 002

Research Ethics

If you have any concerns in regards to the conduct or nature of this research (RO1693), please do not hesitate to contact the Bond University Research Ethics Committee at the following address:

Senior Research Ethics Officer
Complaints
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Bond University
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Telephone: (07) 5595 4194 Fax (07) 5595 1120 Email: buhrec@bond.edu.au

Research Inquires

If you have any queries or would like to be informed of the aggregate research finding, please contact:

Student Investigator: Mr. Brookes Folmli

Signature: ……..

Mobile: 0432 102 778

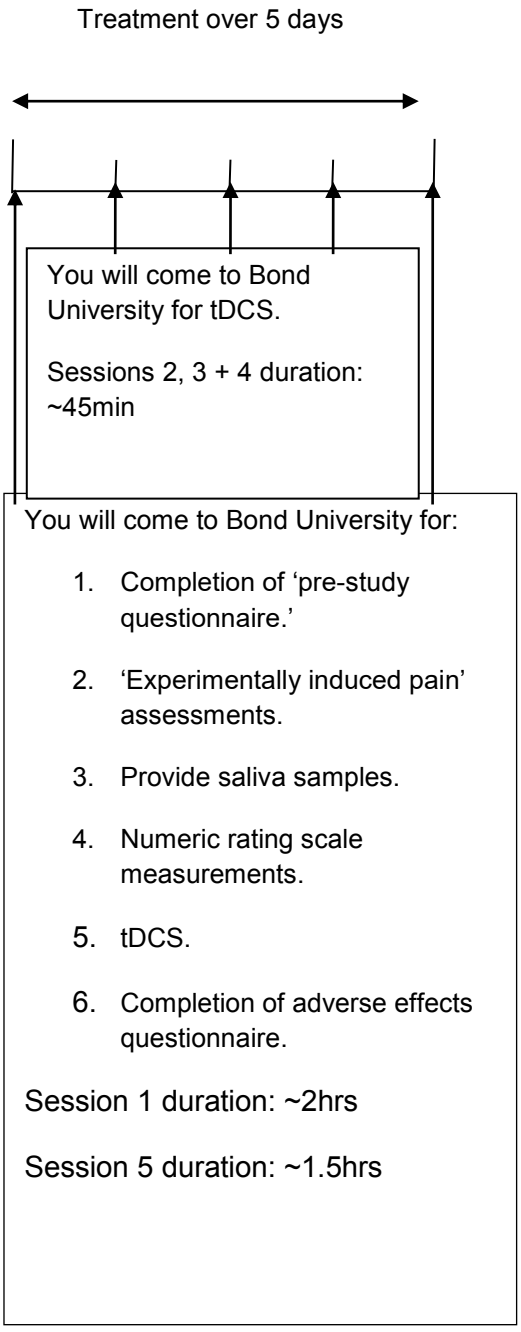
Investigator: Assistant Professor Allan Abbott

Signature: ……

Telephone: 5595 4449

Address: School of Health Sciences & Medicine, Bond University 4229

Timeline of events



Appendix 5 Study 2 pre-study questionnaire

Pre-study questionnaire

Please read the following items carefully and put a tick (✓) on the answer that best corresponds to your current situation. Please be advised that the responses you provide for this questionnaire will be kept strictly confidential and will be used only for the purposes of this research. Therefore, please answer as honestly as you can. If you have questions please do not hesitate to ask the investigators: Brookes Folmli (bfolmli@student.bond.edu.au) and Allan Abbott (aabbott@bond.edu.au).

1. Subject Details	
Name	
Mobile number	
Emergency contact (number, name + relationship to subject)	
Age	
Date form filled	
Gender	Male ☐ Female ☐
Handedness	Right ☐ Left ☐

2. Eligibility	
If you tick yes to any of the following questions in Part A, you will be excluded from the study:	
PART A:	
Do you have any metallic or magnetic pieces inside your brain/skull (except titanium) (e.g. splinters, clips)?	Yes ☐ No☐
Do you have any implanted metal devices that you are aware of (e.g. cochlear, pacemaker, medication pumps, and neuro-stimulators such as deep brain stimulators)?	Yes ☐ No☐
Do you have epilepsy or have ever experienced a convulsion or seizure?	Yes ☐ No☐
Are you someone who consumes heavy amounts of alcohol (e.g. +4 standard drinks/day) very regularly?	Yes ☐ No☐
Have you had any recent or severe heart disease?	Yes ☐ No☐
Are you pregnant or is there any chance you might be?	Yes ☐ No☐
If you tick yes to any of the following questions in Part B, an individual evaluation of your eligibility will be determined:	
PART B:	
Do you have or have ever experienced any focal or generalized neurological condition (e.g. tumour, stroke, encephalitis, severe head trauma, fainting spells)? – if **other** please specify in the available space underneath the boxes	Yes ☐ No☐

Have you ever been involved in a transcranial direct current stimulation (tDCS) study?	Yes ☐ No☐
Do you currently have hair extensions and/ or very thick and course hair?	Yes ☐ No☐
Do you have any skin condition (e.g. eczema), which is located on the scalp and/or forehead?	Yes ☐ No☐

Please list any medication you are taking (or have recently withdrawn from) in the space below:

Appendix 6 Study 2 informed consent

BOND UNIVERSITY
BRINGING AMBITION TO LIFE

FACULTY OF HEALTH SCIENCES AND MEDICINE
Bond University
Gold Coast, Queensland 4229
Australia
Toll free 1800 753 855
(within Australia)
Ph: +61 7 5595 4400
Fax: +61 7 5595 4122
(from overseas)
Email: hsm@bond.edu.au

Statement of Consent

Bond University Human Research Ethics Committee Protocol Number: RO1693

I agree to participate in the above Bond University study. I fully understand that my participation in the study is voluntary only, and can withdraw from the experiment at any time. I also recognise that I will not be identified in any reports on the project, nor to any other party. I have also read the attached explanatory sheet and am fully aware of all associated aims, procedures and risks involved with the investigation. Therefore, I am willing to:

- Provide my age and handedness to be used in future reports and publications in a non-identified form,
- Provide a series of 'experimentally induced pain' measurements,
- Provide saliva samples
- Complete a series of numeric rating scales,
- Allow the researchers to perform transcranial direct current stimulation (tDCS) on me,
- Complete an adverse effects questionnaire
- Have my results of all assessment items be used in future reports and publications in a non-identified form.

Name (please print): .....

Signature: .....

Date: ……..

I (full name of parent/ guardian and relationship to child):

……..

understand the aims, procedures and risks involved with the investigation and

give permission for (full name of child): ……

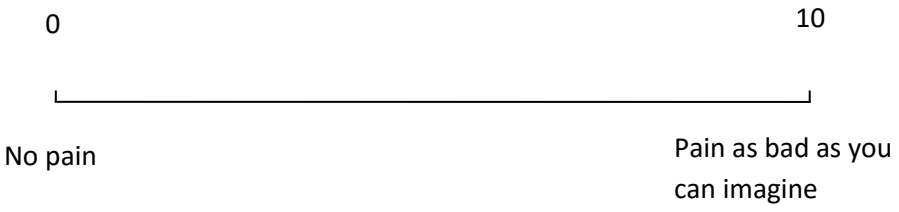
to participate in the RO1693 research.

Signature: .....

Date: ……..

Appendix 7 Study 2 pain visual analogue scales

Place a vertical mark on the lines above to indicate the intensity of pain when you first perceived the stimulus as being ‘painful.’



Place a vertical mark on the lines above to indicate the intensity of pain when you could no longer tolerate the stimuli.

Appendix 8 Study 2 brief sensation test protocol

Sensation protocol

Ask subject to put on blindfold.

Ask subject to rest hands on a table.

Apply stimuli (i.e. soft or sharp object) to subject's hands in 3 anatomical locations: 1) thenar eminence, 2) index finger and 3) midway down pinky side on both sides.

Apply stimuli in mixed order (left vs. right, sharp vs. soft)

Be consistent in stimuli application (i.e. stimuli intensity, angle)

Ask the subject to verbally state when you touch them with either a soft or sharp object

(e.g. sharp right, soft left).

Ask the subject to verbally state if **significant** differences are felt between left and right sides.

Note differences.

Ask subject to scale the differences in intensity (i.e. **compared to dominant**) if significant differences in intensity between left and right sides are detected on a -10 to 0 to +10 scale for each location significant differences in intensity between left and right sides are detected.

10 9 8 7 6 5 4 3 2 1 0

0 1 2 3 4 5 6 7 8 9 10

Appendix 9 Study 2 participant blinding visual analogue scales

Visual Analogue Scale (participant blinding)

Do you feel that you have just received the 'real' stimulation intervention?

Yes ☐

No ☐

Not confident at all completely confident

Place a vertical mark on the line above to indicate the level of confidence you have in your answer.

Appendix 10 Study 2 scalp stimulation adverse effects questionnaire

Side effects	Number of occasions
Itching sensation	
Tingling sensation	
Burning sensation	
Local pain sensation	
Visual sensation (i.e. flash of light)	
Headache	
Skin irritation	
Nausea	

Appendix 11 Study 2 self-report general pain sensitivity questionnaire

Self reported measure of pain sensitivity questionnaire

The questionnaire contains a series of questions in which you should imagine yourself in certain situations. You should then decide if these situations would be painful for you and if yes, how painful they would be on a scale from 1-10 where 0 is no pain; and 10 the most severe pain that you can imagine.

Imagine you bump your shin badly on a hard edge, for example, on the edge of a glass coffee table.	
Imagine you burn your tongue on a very hot drink.	
Imagine your muscles are slightly sore as the result of physical activity.	
Imagine you trap your finger in a drawer.	
Imagine you take a shower with lukewarm water.	
Imagine you have mild sunburn on your shoulders.	
Imagine you grazed your knee falling off your bicycle.	
Imagine you accidentally bite your tongue or cheek badly while eating.	
Imagine walking across a cool tiled floor with bare feet.	
Imagine you have a minor cut on your finger and inadvertently get lemon juice in the wound.	
Imagine you prick your fingertip on the thorn of a rose.	
Imagine you stick your bare hands in the snow or ice for a couple of minutes or bring your hands in contact with snow or ice for some time, for example, while making snowballs.	
Imagine you shake hands with someone who has a normal grip.	
Imagine you shake hands with someone who has a very strong grip.	
Imagine you pick up a hot pot by inadvertently grabbing its equally hot handles.	
Imagine you are wearing sandals and someone with heavy boots steps on your foot.	
Imagine you bump your elbow on the edge of a table (''funny bone").	
Finally, ''How do you rate your (bodily) pain sensitivity compared with that of the average persons" on a scale of 0–10, 5 is the same as the average person, under 5 is lower than the average person and above 5 is higher than the average person. Therefore 0 means ''very much lower" and 10 means ''very much higher".	